KINETIC MODELING AND ASSESSMENT OF LIME PRETREATMENT OF POPLAR WOOD

A Dissertation

by

ROCIO SIERRA RAMIREZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2010

Major Subject: Chemical Engineering

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Approved by:

Chair of Committee,	Mark T. Holtzapple
Committee Members,	Sam M. Mannan
	Charles J. Glover
	Cady R. Engler
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ABSTRACT

Kinetic Modeling and Assessment of Lime Pretreatment of

Poplar Wood. (December 2010)

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Because of widespread availability, low cost, sustainability, and potential supply far greater than that of food crops, lignocellulosic biomass is one of the most promising feedstocks for producing biofuels through fermentation processes. Among lignocellulose choices, poplar wood is appealing because of high energy potential, above-average carbon mitigation potential, fast growth, and high yields. Lignocellulose structural features limit accessibility of enzymes or microorganisms. To overcome these limitations, pretreatment is required. Among several choices of pretreatment, lime pretreatment is preferred because lime is the cheapest alkali, safest to handle, easy to recover, and compatible with oxidants.

The main effect of lime pretreatment is to degrade lignin, which occurs with good carbohydrate preservation and is enhanced with oxidants. Among several choices of oxidant, oxygen and air are preferred because of low cost and widespread availability.

This study systematically assesses the effects of lime pretreatment on poplar wood using four different modes: long-term oxidative, long-term non-oxidative, short-term constant pressure, and short-term varying pressure. Long-term pretreatments use temperatures between 25 and 65°C, air if oxidant is used, and last several weeks. Short-term pretreatments use temperatures between 110 and 180°C, pressurized oxygen, and last several minutes to hours.

Pretreatment was assessed on the basis of 3-day enzymatic digestibility using enzyme loadings of 15 FPU/g glucan in raw biomass. The results were used to recommend pretreatment conditions based on highest overall yield of glucan (after combined pretreatment and enzymatic hydrolysis) for each pretreatment mode.

For each pretreatment mode, kinetic models for delignification and carbohydrates degradation were obtained and used to determine the conditions (temperature, pressure, and time) that maximize glucan preservation subjected to a target lignin yield. This study led to conclude that the most robust, and selective mode of lime pretreatment is varying pressure.

To God The Wonder of All Wonders

To my family that always supported me and gave me the strength and the reason to WRAP IT UP

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Last but not least, I would like to especially thank my parents, Aurora and Antonio for giving me and my family continuous and unconditional support and encouragement throughout graduate school. Thanks to them for their never ending love.

NOMENCLATURE

a_{ij}	Frequency factor
$\beta_{\iota\varphi}$	Exponent
C_i	Component *i* content at time *t*
C_{i0}	Component *i* content at time zero
CP	Short-term constant pressure pretreatment
E_{ij}	Activation energy
F_c	Calculated statistic F
i	Index. *L* for lignin, *G* for glucan, and *X* for xylan
j	*f* and *s* (Model 1) and *f*, *m*, and *s* (Model 2)
k_{ij}	Rate constant
LTN	Long-term non-oxidative pretreatment
LTO	Long-term oxidative pretreatment
M	Oxygen molecular weight (32 kg/kmol)
M_{O2}	Initial oxygen charge for VP pretreatment
p	Number of parameters in the model
P_{O2}	Oxygen pressure
R	Ideal gas constant (8.314×10^{-3} kJ/(mol·K))
n	Number of experiments
S_{dG}	Differential glucan selectivity
S_{dX}	Differential xylan selectivity

S_G	Integral glucan selectivity
S_X	Integral xylan selectivity
T	Temperature
T_r	Absolute room temperature (298 K)
V	Free reactor volume after filled with biomass, water, and lime ($8.30\times10^{-5}\,m^3$)
VP	Short-term varying pressure pretreatment
Y_i	Pretreatment yield of Component *i* at time *t*
y_i	Measured data
$\hat{y}_i$	Estimated value of dependent value
Y_{ij}	Yield of Component *i* at time *t*
Y_{ij0}	Yield of Component *ij* at time zero
Y_T	Total solids pretreatment yield at time *t*

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INTRODUCTION

In the foreseeable future, the world's energy supply is vulnerable to failure arising from environmental catastrophe to sudden supply interruptions. Some driving forces for this reality are worldwide population growth and limited availability of low-cost feedstocks. Clearly, alternatives to fossil fuels must be implemented to support reliable, affordable, clean, and domestic energy and chemicals production.

Ethanol produced from corn grain and diesel produced from soybean are currently the predominant transportation biofuels in the United States.[1] Globally, the United States (using corn grain) and Brazil (using sugarcane juice) are the two primary producers of bioethanol with 17,000 million US liquid gallons per year produced in 2009 accounting for about 89% of the world production.[2] These paths to ethanol production are often criticized because of significant requirements for arable land and water, important environmental impacts, and competition with food resources.

To replace these food-based resources, intensive work has been done to identify alternative feedstocks and the corresponding conversion processes. Lignocellulosic feedstocks are promising due to their potential supply far greater than food crops. Decisive, widespread industrial utilization of lignocellulosics has been impeded by high inertia, lack of price competiveness with petroleum based fuels, and because lignocellulosics are recalcitrant to fermentation.

This dissertation follows the style of *Biotechnology Progress*.

Compared to sugar and starch feedstocks, lignocellulose poses a challenge in technological and economical terms. Table 1 presents routes for lignocellulosic processing that are industrially applied or are in mature research stage. Although a particular lignocellulosic biomass may have explicit benefits for a specific route, the amount of energy potentially available from it is the same regardless the process.[3] A comparison among three process with different intermediates shows the highest yield for the carboxylate route.[3]

The chosen route will determine (1) the actual amount of energy recovered, (2) the form of that energy, (3) environmental impacts, and (4) the costs associated with the production process. Even with highly efficient processes, there is extensive debate on whether biomass sources would be sufficient to significantly contribute to meeting demand for energy, while also accomplishing important objectives, such as food production, preservation of wilderness (environment), and recreation.[1, 4, 5] A study conducted by Berndes et al.[6] illustrates this point (Figure 1). This study is based on a review of 17 earlier studies on the subject identified in the plot by the last name of the first author. The solid and dashed lines correspond to predictions whose corresponding studies are identified on the right of the line. The approximate global primary energy consumption of the year in which the paper was written (2003) is included for comparison (about 420 EJ/year). The major reason for the differences in results and predictions is that the two most crucial parameters—land availability and yield levels in energy crop production—are very uncertain, and subject to widely different opinions.

The author of the present work is convinced that all of the primary objectives of biomass are achievable including meeting important demands for energy production if the following policies are implemented:

1. Enhanced plant species resulting from genetically engineered crops, improved breeding techniques and hybrids. These practices allow for use of marginal lands, cultivation in extreme climates, and in aquatic environments.
2. Implementation of techniques to avoid negative impacts on soil quality.
3. Improved crop productivity obtained through better cultivation techniques.
4. Implementation of a sustainable and robust system for recollecting wastes and residues (municipal, industrial, and agricultural).
5. Appropriate selection of feedstock and conversion technology.
6. Improved efficiency of the production technology obtained through intensive research and development.
7. More efficient use of energy, including vehicle efficiency.

The aim of the present work is to contribute to point six in this list by recommending methods and conditions of pretreatment that would render poplar wood digestible to fermentation processes.

Table 1. Diverse alternative current technologies to produce bioethanol, other fuels and chemicals.

Feedstock	Process I[(1)]	Intermediate	Process II[(2)]	Product	Research Institute	Company
Sugar Crop	Extraction	Sugar	Fermentation	Alcohol	-	Cosán
Starch crop	Amylasas hydrolysis	Sugar	Fermentation	Alcohol	-	Nebraska Energy
Lignocellulose	Fast pyrolysis	Bio-oil	Hydrogenolysis	Hydrocarbon	Iowa, Georgia Tech, NREL	
Lignocellulose	Gasification	CO/H_2	Fisher Tropsch	Hydrocarbon	NREL	Range Fuels
Lignocellulose	Gasification	CO/H_2	Catalytic reaction	Alcohol		Standard Alcohol Co. Power Energy Fuels
Lignocellulose	Gasification	CO/H_2	Fermentation	Alcohol		Alico Inc., Bioenergy, Coskata
Lignocellulose	Acid Hydrolysis	Sugar	Fermentation	Alcohol		Arkenol, BlueFire Ethanol, Masada
Lignocellulose	Enz. hydrolysis	Sugar	Fermentation	Alcohol	NREL, UCRS	Abengoa, Celunol, Mascoma, Iogen
Lignocellulose	Enz. Hydrolysis	Sugar	Fermentation	Hydrocarbon		Codexis, Amyris, LS9
Lignocellulose	Enzymatic hydrolysis	Sugar	Chemical	Hydrocarbon	Wisconsin	Virent
Lignocellulose	Enzymatic Hydrolysis	Carboxylate	Chemical	Alcohol Ester, Ether, Hydrocarbon	Texas A&M Maine	Terrabon
Lignocellulose	Enzymatic Hydrolysis	Sugar to carbox. acid	Chemical	Alcohol Ester, Ether, Hydrocarbon	Cornell	ZeaChem

(1) Main process from feedstock to main intermediate. (2) Main process from intermediate to goal product

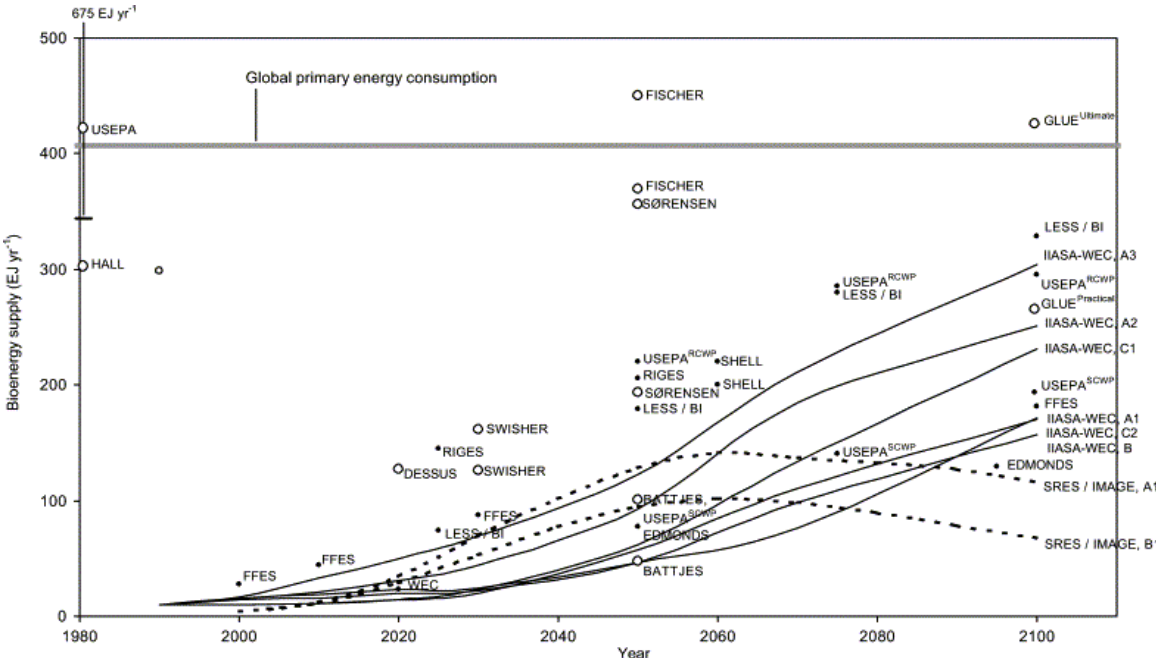


Figure 1. Potential biomass supply for energy over time.[6]

Lignocellulosic feedstocks

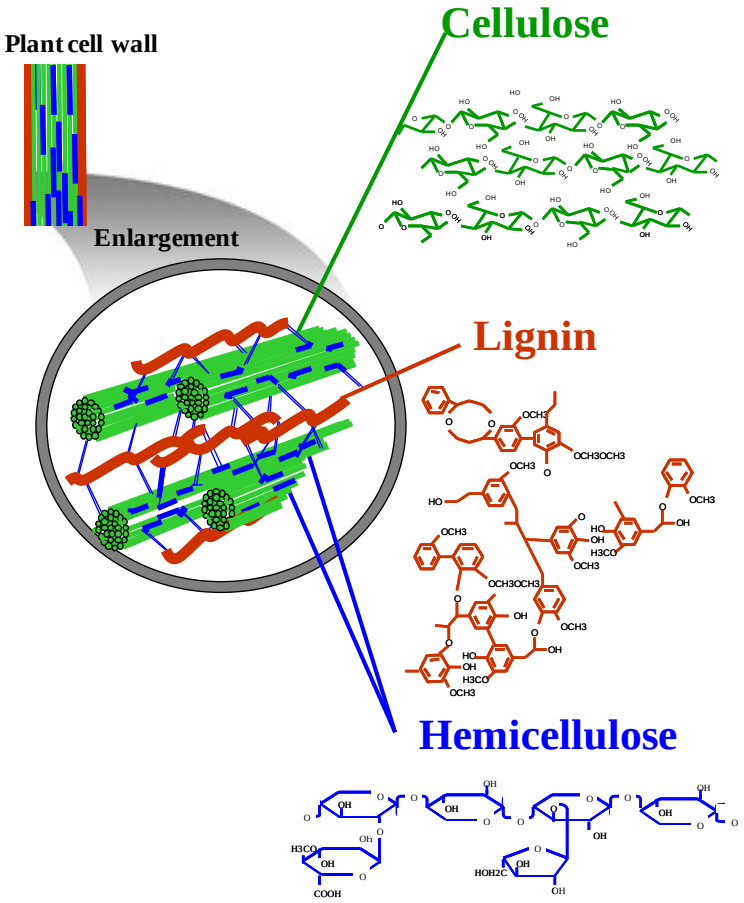
Lignocellulosic biomass can be derived from diverse, inexpensive, and widespread resources, e.g., forestry resources or residues, agricultural residues, food scraps, municipal solid waste, and energy crops. Some of these resources have no better alternative use. Based on favorable carbohydrate content, high crop yields, national interest, and ability to produce liquid fuels via fermentation, this study focuses on lignocellulosic material derived from forestry and agricultural products and residues. Specifically, this study assesses poplar wood because it grows on marginal lands and requires minimal fertilization. It may be mechanically harvested, and is easily propagated from either stem cuttings or tissue culture. There has been some interest in using poplar as an energy crop because of its high energy potential,[7] carbon mitigation

potential,[19, 20] fast growth, and high yields.[8] Its biochemical conversion to ethanol has been the subject of other studies.[9-11]

Lignocellulose composition

Every biomass is unique in its chemical and physical properties; however, generalizations can be made regarding the plant cell wall. It can be described as a macromolecule composed of cellulose fibers embedded in a covalently joined matrix of lignin and hemicellulose.[12] These three structural components (lignin, cellulose and hemicellulose) are not uniformly distributed in cells, and their relative mass proportions can vary widely depending on the specific plant, morphological region, and age.[13] On average, these structural substances represent 90% of the dry weight of most plants.[14] Other polymeric constituents (e.g., pectin, starch and proteins) may be present in lesser and varying quantities. Low-mass compounds, such as extractives, may also be found. Although cellulose composition is identical in all plants, the structure and composition of hemicellulose and lignin are unique to plant species. Figure 2 presents a scheme of plant cell wall and the chemical structure of cellulose, hemicellulose, and lignin. A brief explanation follows:

Cellulose is a linear, unbranched polymer of anhydroglucose connected by β-1,4 linkages. Native cellulose occurs as densely packed, hydrogen-bonded elementary fibrils of pure cellulose embedded in a matrix of hemicellulose. Native cellulose is insoluble and contains both crystalline and amorphous regions. This complexity makes cellulose resist enzymatic hydrolysis without prior pretreatment.[15]



38-50%

✓Most abundant and important biopolymer
✓Composed of anhydroglucose connected by β-D-glucopyranose units linked together by (1-4) glycosidic linkages.
✓Good feedstock for alcohol production
✓Due to high crystallinity is relatively inert to chemical treatment and insoluble in most solvents

15-30%

✓Largest non-carbohydrate fraction of lignocellulose
✓Highly cross-linked polymer. The monomeric units are p-hydroxycinnamyl alcohols
✓Covalently bound to hemicellulose
✓High energy content
✓Resists biochemical conversion

23-32%

✓The second major naturally occurring carbohydrate-based polymer
✓Built from hexoses, pentoses and deoxyhexoses (mostly xylose)
✓Small amounts of uronic acids are also present
✓May be used as feedstock for biochemical conversion

Figure 2. Main components in plant cell wall.[16]

Hemicellulose consists of short, branched chains of sugars (three hexoses: D-glucose, D-galactose, and D-mannose and two pentoses: D-xylose and L-arabinose) and modified sugars such as xylan with acetyl groups at the C_2 and C_3 positions. Hemicellulose is amorphous because of its highly branched nature. Because of the amorphous morphology, hemicelluloses are partially soluble or swellable in water.[17]

Lignin is a highly cross-linked polymer built from phenylpropane units covalently bound to hemicellulose. The monomers are trans-coniferyl alcohol, trans-sinapyl alcohol, and trans-p-coumaryl alcohol[18](Figure 3). The molar distribution of these units varies widely with biomass type. The first two units dominate softwoods and hardwoods, respectively, whereas the coumaryl unit is primarily found in grasses.[19] Nevertheless, the greatest molar fraction of monomers contain one phenolic hydroxyl group, which may be free or bound (as aryl ether).[20] Two monomers can be connected by only one linkage, regardless of the number of bonds between them (Figure 4).

The units are linked monofunctionally and bifunctionally to form linear (primary) chains. Additionally, nonterminal units in two linear chains may be cross-linked to form a tree like polymer (Figure 5).

Cross-linked units have functionality of three. Four functional groups are possible but very unlikely due to stereochemistry.[21] All of these features of the lignin polymer are important when discussing delignification and oxygen delignification processes and even more important for modeling delignification. All of these topics will be discussed in further sections of this work.

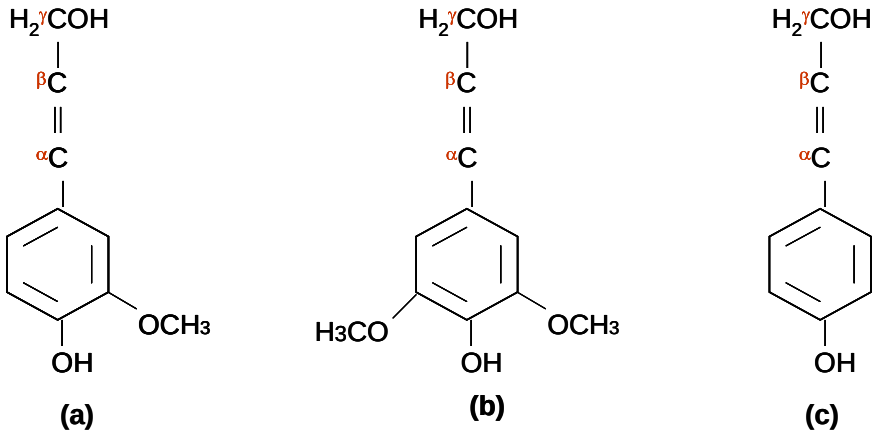


Figure 3. Lignin monomers.[18] (a) Trans-coniferyl alcohol (b) Trans-sinapyl alcohol (c) Trans-p-coumaryl alcohol.

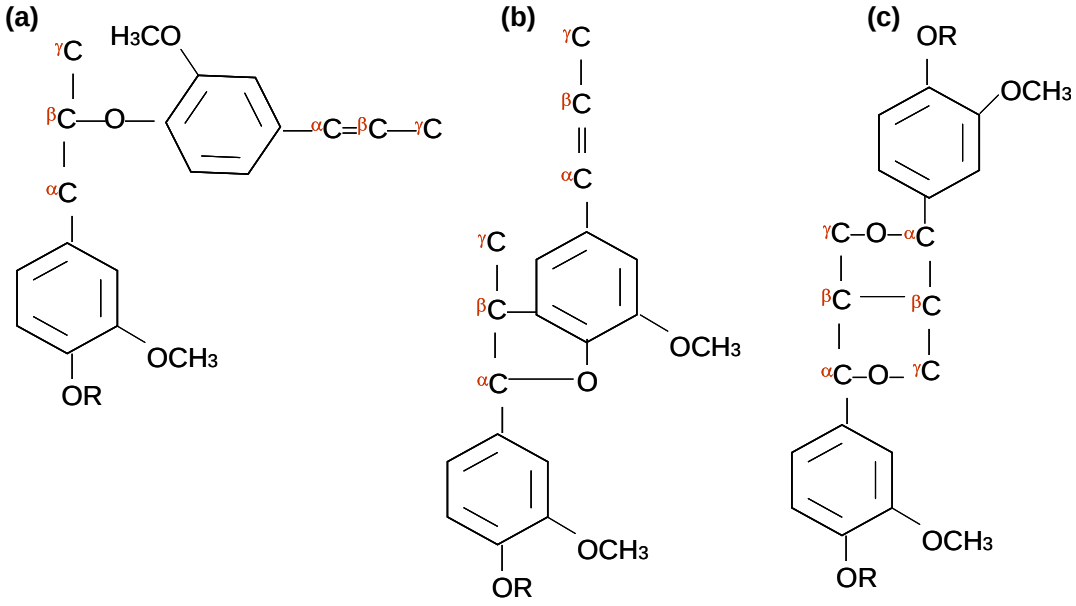


Figure 4. Phenylpronane linkages.[21, 22] (a) one bond: β-aryl ether (b) two bonds: one α-aryl ether and one β-aryl (c) three bonds: two aryl ether and one aryl aryl. All three are examples of "one" linkage.

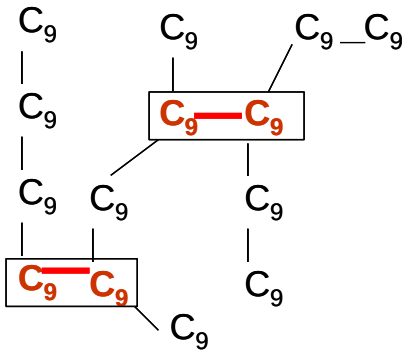


Figure 5. Cross linked units with three functionalities.[22] In this figure C_9 denotes a phenylpropane monomer in lignin.

Structural features of biomass that hinder digestibility

The accessibility of enzymes or microorganisms to lignocellulosic biomass is limited because of biomass structural features. Characterizing, understanding, and overcoming these limitations is essential to develop processes based on fermentation routes; however, the mechanisms by which each of these features affect digestiblity have not been completely elucidated and are the subject of extensive debate. Uncertainty results because experimental manipulation of one feature inevitably affects others. Some important points in the discussion are summarized below.

High lignin content. When closely associated with cellulose microfibrils, lignin blocks the access to the carbohydrate fraction of biomass. Delignification – disruption of lignin structure by hydrolysis and/or degradation– swells biomass and increases internal surface area and median pore volume, thereby improving digestibility.[23-26] The extent of delignification required to enhance digestibility differs depending on the feedstock. Some affirm that reducing biomass lignin content below ~10% will not

further improve biodegistibility.[24] However, others state that there is no clear correlation between the hydrolysis yield and lignin content.[27] Nevertheless, most researchers assert that if not the absolute amount of lignin, its location and chemical/physical structure affect the enzymatic/fermentation yields.[25, 28, 29]

Hydrolysis of hemicellulose. With little or no lignin degradation, at least 50% hemicellulose significantly increases digestibility.[30-32] This route is advantageous because the lignin polymer is recovered in non-soluble form, which avoids the formation of soluble lignin degradation products that may inhibit fermentation. Also, the potential energy recovery from lignin through combustion and/or gasification routes is higher.[29] However, some researchers state that residual lignin is undesirable because lignin is a competitive cellulase adsorbent.[33 34] Additionally, lignin degradation products may inhibit subsequent enzymatic steps. Hemicellulose and lignin are covalently linked, thus hemicellulose hydrolysis affects lignin as well.[35]

Presence of acetyl groups on hemicellulose. In native plant cells, xylan backbones are acetylated (CH_3COO^-) with about 70% of xylan residues containing acetyl groups.[17] Several studies have shown that removing acetyl groups from xylan, enhances biomass digestibility through increased swelling.[23, 24 31]

High cellulose crystallinity. Cellulose microfibrils have both crystalline and amorphous regions. Crystallinity is affected by the relative amounts of these two regions. Cellulase readily hydrolyzes the more accessible amorphous portions of cellulose. In contrast, it takes a tighter binding between enzyme and substrate to effectively hydrolyze crystalline cellulose;[36] thus, it is expected that reducing biomass

crystallinity will improve digestibility. Nevertheless, researchers have reported conflicting results regarding the relationship between biomass digestibility and crystallinity.[23, 24, 37, 38] Rather than crystallinity per se, this variability may result from non-related factors such as differences in drying conditions, general methods of substrate preparation prior to crystallinity measurement, and conflicting results on the change of crystallinity during hydrolysis arising from the presence of residual cells/protein in the sample.[39]

Degree of polymerization of cellulose. The degree of polymerization (DP) is defined as the number of glucosyl residues per cellulose chain. It determines the relative abundance of terminal and interior β-glucosidic bonds, and of substrates for exo-acting and endo-acting enzymes.[39] Exoglucanases act on chain ends and thus decrease DP only incrementally; however, they have a marked preference for substrates with lower DP.[40] Endoglucanases act on interior portions of the chain and thus rapidly decrease DP leading to cellulose solubilization, which may favor digestibility; however, no irrefutable conclusion has been drawn about the importance of DP in determining hydrolysis rates of pretreated cellulosic biomass. [32, 38, 41-43]

Surface area and pore volume. Cellulase enzymes must bind to the surface of substrate particles before hydrolysis of insoluble cellulose occur. The maximum amount of protein that can be adsorbed during enzymatic saccharification of cellulose glucose is a controlling factor for hydrolysis rates and yields, and directly depends on enzyme accessibility to active sites on the solid substrate.[44] Several techniques have been applied to measure surface area and pore volume, for example, the BET method uses absorbed

nitrogen molecule and the method of Stone and Scallan excludes solutes.[45] The effectiveness of these methods has been criticized because they potentially overestimate the effective cellulase accessible area.[46] Alternatively, measurements can be made using X-ray scattering (SAXS) and mercury porosimetry. However, because the ultimate goal of measuring surface area and pore volume is to estimate the enzyme accessibility, a more accepted technique determines protein adsorption onto the substrate by calculating the difference between the total amount of protein initially added and the amount left in solution (or in the solids) at any time of hydrolysis.[44] To measure protein content, several methods are applied: colorimetric methods such as BCA, Bradford, and Lowry,[47-49] protein precipitation by acetone,[50] and the Dumas method,[51] which requires an estimation of the nitrogen factor to translate nitrogen readings into protein content. No contradictory opinions were found in the literature regarding the direct relationship between enzyme adsorption and biomass digestibility.

Pretreatment

Pretreatment is the first step in processes that convert lignocellulosic feedstocks to sugar or carboxylate intermediates, which are then converted into liquid fuels.[21] Figure 6 shows flow diagrams of two of these processes: the MixAlco process (which gives the highest yields[52]) and traditional sugar fermentation (highly favored in the United States).

Pretreatment is required to overcome lignocellulose recalcitrance. Its goal is to alter biomass structural features to make it more digestible.

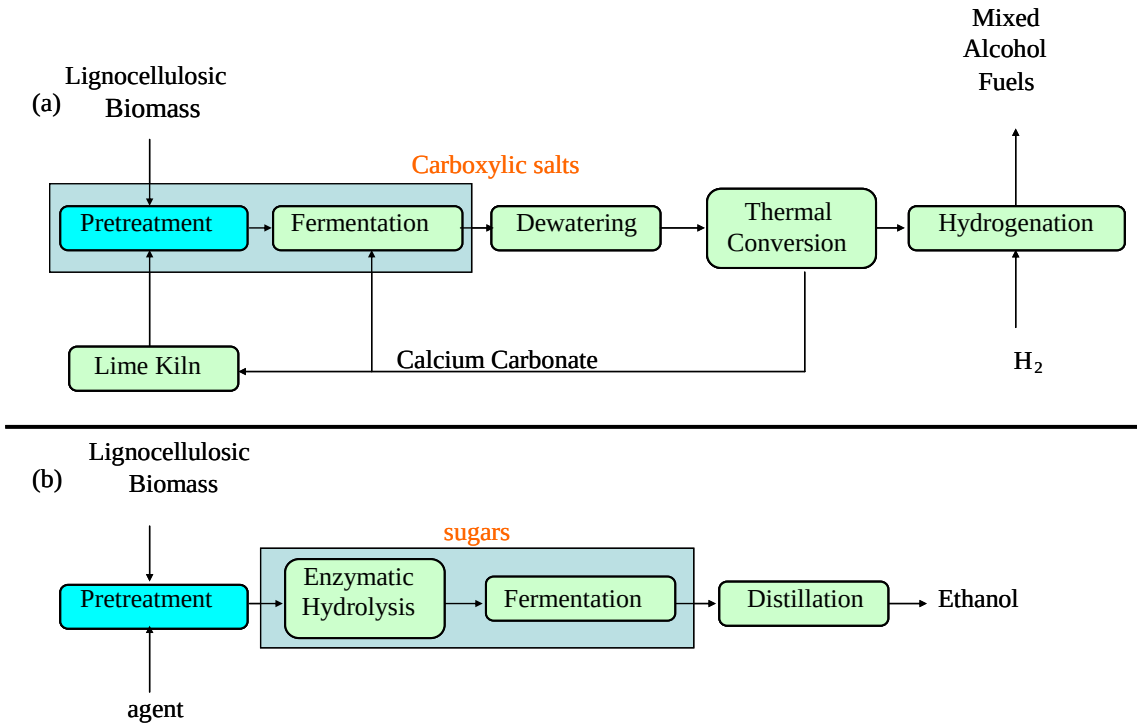


Figure 6. Flow diagram of two routes for ethanol production. (a) MixAlco process to obtain a mixture of alcohol fuels (b) Traditional process which uses enzymatic hydrolysis of sugars. Shadow boxes indicate two stages that need only one reactor.

During biomass pretreatment, its macroscopic and microscopic size, structure and chemical composition are affected (Figure 7).[23, 31, 39, 53, 54]

Major effects of pretreatment are reduction of lignin content, hydrolysis of hemicellulose fraction, decrease in crystallinity, removal of acetyl in hemicellulose, reduction of degree of polymerization, and increased surface area and pore volume.

As a result of pretreatment, hydrolysis of the carbohydrate fraction is achieved more rapidly and with yields that are greatly improved compared to untreated biomass. Typically, hydrolysis yields in the absence of pretreatment are <20% of theoretical, whereas yields after pretreatment often exceed 90% of theoretical.[29]

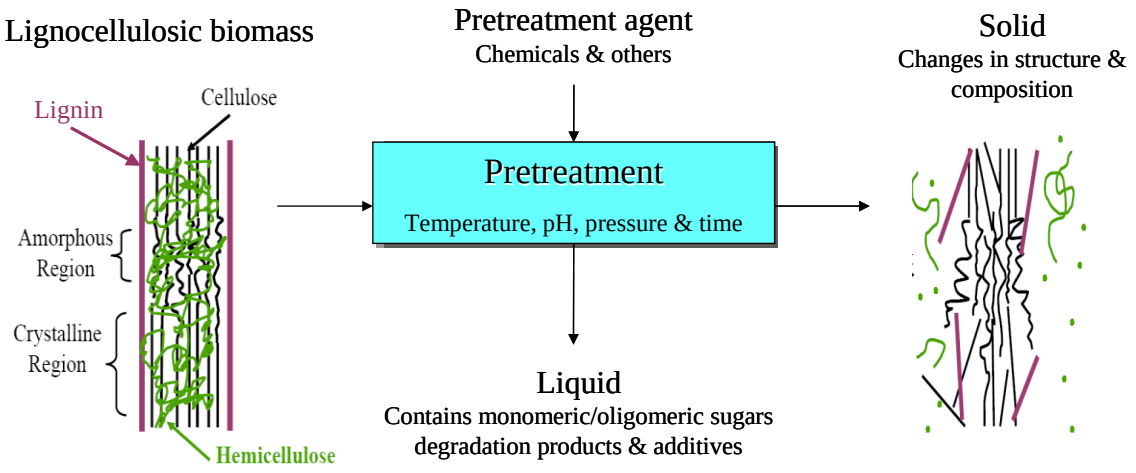


Figure 7. Effects of pretreatment on lignocellulosic biomass.

The mechanisms by which pretreatments act are not well understood; however, it is known that different pretreatments affect biomass in very different ways.[31] Currently available pretreatment methods are biological (biological agents), chemical (chemical catalyst such as acids, bases, or cellulose solvents), and physical (mechanical size reduction, explosion, decrystallization, compression, radiation, and hydrothermolysis).

An ideal pretreatment will retain nearly all the cellulose present in the original material so that nearly theoretical yields are obtained from enzymatic hydrolysis or fermentation. Other desirable features of pretreatment follow: (1) Generate high hemicellulose yields. Because the hemicellulose content in biomass is high, the potential yield from this fraction significantly affects process efficiency and economy. (2) Limit the formation of degradation products that may inhibit fermentative microorganisms. (3) Minimize energy demand. (4) Be effective on multiple lignocellulosic feedstocks. (5) Minimize costs. Pretreatment has a major influence on overall process cost because of its

own cost and because it directly affects other operations.[29, 55, 56] Because it is the most costly step in the traditional process to produce ethanol, pretreatment has been the subject of active research and development (Figure 8). Technical advances are needed for overall costs to compete with conventional commodity fuels and chemicals.[57]

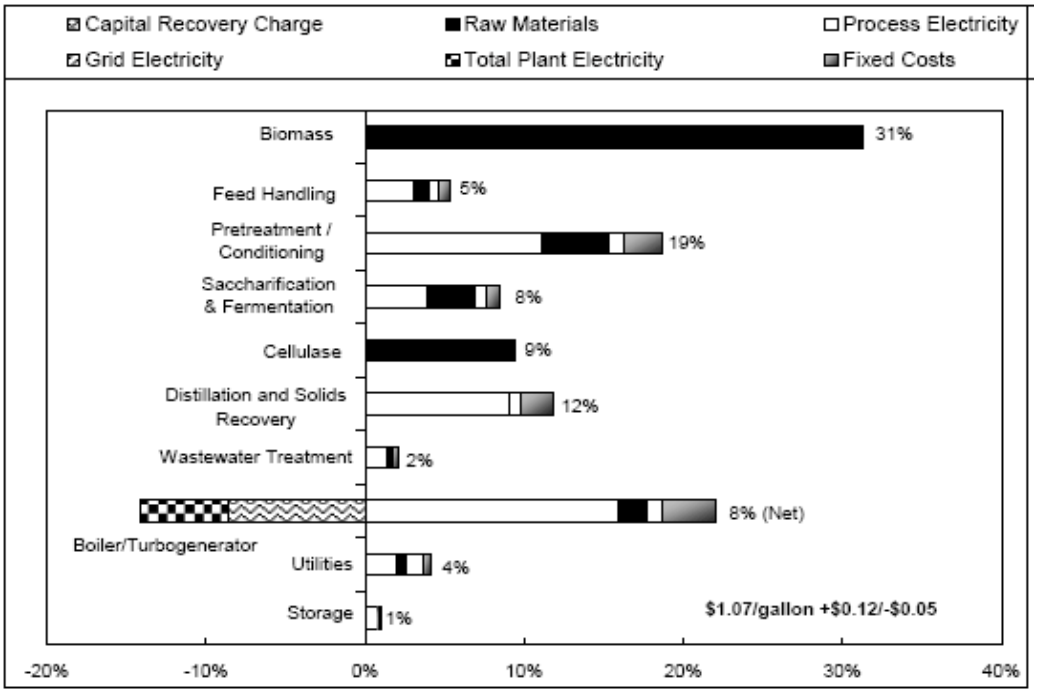


Figure 8. Cost contribution details from each process area in an ethanol plant (corn-stover feedstock).[56] The cost is given in % of Ethanol selling price. The process illustrated in this example uses sugar intermediate that is converted to ethanol through fermentation.

Pretreatment conditions and effects

A selected set of pretreatments is explained below. Table 2 summarizes their main effects and conditions. These pretreatments were chosen because they are cost effective and because comparative performance data is available.[31, 58, 59]

Table 2. Selected pretreatments with the corresponding conditions and effects on the lignocellulosic biomass.

Name	Operating conditions	Effects	Advantages	Disadvantages	TSY
Dilute Acid	Temp: ~140–190ºC Pressure: Not reported Time: 5– 30 min Chemicals: H_2SO_4 [~0.5–1.0%] Solids: 16% (Flow through)	Surface Area: I Delignify: N Lignin alter: I CrI: N Hem.removed.: I Deacetyl: ND	• Widely studied for many years. Well developed. • Most species perform well.	• Corrosion: requires expensive materials. • Gypsum formation (if neutralized with inexpensive $Ca(OH)_2$). • Inhibitors (furfural and aldehydes). • Grinding the cellulose to 1 mm a (33% of the power requirements).	Batch 92.4 Flow-through 96.6
Hot Water	Temp: ~180–190ºC Pressure: 350–400psig Time: 15 min Chemicals: None (KOH may be used if pH is to be controlled)	Surface Area: I Delignify: N Lignin alter: ND CrI: N Hem.removed: I Deacetyl: I	• No cost due chemicals/ recovery. • No neutralization and/or conditioning. • Size reduction of biomass not needed. • The cleavage of O-acetyl and uronic acid helps to catalyze pretreatment. • Fermentable liquid hydrolyzate.	• Inhibitors due to acidification of water (furfural and aldehyde). • Variability in results related to the biomass type. • High lignin solubilization impeding recovery of hemicellulose sugars.	pH control flow-through 87.2
Ammonia (AFEX)	Temp: ~60–100°C Pressure: 250–300psig Time: ~5 min Chemicals: Ammonia [1.0 kg ammonia /kg dry biomass] Solids: 60%	Surface Area: I Delignify: I Lignin alter.: I CrI: I Hem.removed: P Deacetyl: I	• Most ammonia can be recovered. • Unrecovered ammonia is used in down- • stream processes by microbial (N_2 source). • There is no wash stream in the process. • Dry process, thus permits much higher solids loadings in the fermentation.	• Herbaceous and agricultural residues are well suited for AFEX but poor performance on hardwoods and not attractive for softwoods.	94.4
Ammonia (ARP)	Temp: ~180°C Pressure: Not reported Time: ~14 min Chemicals: Ammonia [~15%] Flow rate: 1 mL/(cm^2·min)	Surface Area: I Delignify: I Lignin alter.: I CrI: I Hem.removed: P Deacetyl: I	• High and adjustable degree of delignification (70-85% of total lignin). • Rapid delignification reaction. • Retains more than 92% of the cellulose.	• Solubilizes ~50 of hemicellulose. • Must run in two-stage to prevent hemicellulose loss during (hot water treatment is first stage). • High cost of ammonia and ammonia recovery.	89.4
Lime	Temp: ~50–160°C Pressure: 1 atm (air) or 300psi (oxygen) Time: ~2 h to 8 weeks Chemicals: lime [~0.2 g lime/g dry biomass]	Surface Area: I Delignify: I Lignin alter.: I CrI: ND Hem.removed: P Deacetyl.: I	• Preserve sugars. • Limit the formation of degradation. products or inhibitors of fermentation. • Minimize energy demand. • Effective on multiple feedstocks. • Low capital costs (long-term).	• Required pretreatment time is much longer than in any other approaches.	86.8

I: Important. P: poor. ND: Not determined. N: Negative. TSY: Total sugar yield of corn stover at 15 FPU/g glucan in raw biomass (total sugar hydrolyzed/100 g available sugars in raw biomass). CrI: Biomas crystallinity

Dilute sulfuric acid pretreatment is one of the oldest pretreatments and is often favored because of extensive development. There are two types of dilute acid pretreatment: batch and flow-through. In batch pretreatment, the biomass is presoaked in the acid for at least 4 hours at room temperature before pretreatment; then, the acid-biomass mixture is placed in a vessel that can be heated through the vessel walls or by steam injection.[60, 61]

In the flow-through mode, an acid and water mixture is first heated, then injected through a confined layer of biomass. The batch mode has a higher acid concentration and a lower temperature than the flow-through pretreatment.[62]

SO_2 steam explosion uses SO_2 gas and steam as opposed to acid pretreatments that use sulfuric acid and liquid water. At the end of this pretreatment, the pressure is suddenly released causing an explosion.[63]

Liquid hot water pretreatment uses high pressure to maintain water in the liquid state at elevated temperatures. The reactor may be arranged in co-current or countercurrent fashion. A flow-through reactor may also be set by passing hot water through a stationary bed of lignocellulose. During hot water pretreatment, the pH may drop for two reasons: the pKa of water is lowered with increased temperature, and hot water cleaves hemiacetal linkages liberating acids during biomass hydrolysis. A variation of the flow-through hot-water pretreatment uses a base (if required) to maintain the pH between 5 and 7. In this case, the pretreatment is called pH-controlled liquid hot water pretreatment.[62]

Steam explosion pretreatment uses steam instead of hot liquid water and ends with a rapid decompression attained by suddenly releasing the pressure.[64]

Ammonia pretreatment may occur in either one of two ways: batch and flow-through. In the batch mode (Ammonia Fiber Explosion, AFEX*)* the lignocellulosic materials are exposed to liquid ammonia at high temperature and pressure for the required pretreatment time. Then, the pressure is suddenly released causing rapid vaporization of the ammonia, and the material explodes. Most of the ammonia can be recovered for re-use (up to 99%).[65-67] In the flow-through mode (Ammonia Recycled Percolation, ARP) aqueous ammonia solution is fed to a column reactor packed with biomass. Ammonia is separated and recycled.[68] Batch pretreatment requires higher ammonia concentration and lower temperature than the flow-through pretreatment.[31]

Lime pretreatment has proven to be a useful method for selectively reducing the lignin content of lignocellulosic biomass without significant loss in carbohydrates, thus realizing an important increase in biodigestibility.[11, 16, 69, 70] In lime pretreatment, the biomass is pretreated with calcium hydroxide and water under different conditions of temperature and pressure.

Comparative pretreatment studies

Comparisons between different pretreatment methods have been difficult and inconclusive because of notable differences in feedstocks, methods to assess digestibility and different ways to report results. Wyman et al.[71] obtained exceptionally meaningful

comparisons between different pretreatment methods (the selected pretreatments included in

Table 2) because of the uniformity imposed to the study: same analytical procedures, same feedstock source (corn stover), and same cellulase source. Additionally, experiments were performed by researchers with notable R&D in a particular pretreatment. This was possible through the participation of five universities in North America and the National Renewable Laboratory within the group denominated the Biomass Refining CAFI (Consortium for Applied Fundamentals and Innovation). More details on the logistics have been explained by Wyman et al.[59]

From these studies, it was possible to determine the total sugar yields obtained after combining pretreatment and enzymatic hydrolysis (

Table 2). Assessed in this way, lime pretreatment showed very competitive yields. These yields and pretreatment conditions were used by Eggeman and Elander[58] to determine capital investments for the different pretreatment options using ASPEN® simulations. These comparisons showed lime pretreatment has the lowest total fixed capital investment.[58]

The second part of this systematic and methodic study, compared six pretreatment modes for poplar wood and the results of this comparisons have been published. This study is part of this major project.

Lime pretreatment background

The use of lime is advantageous because lime is inexpensive (the lowest cost alkali); it is easy to recover,[72] which makes it cost-effective and environmentally

friendly; it is safe to handle; and it is compatible with oxidants. This feature is important because the main effect of lime pretreatment is lignin removal, which is significantly enhanced by the presence of an oxidative agent.

Features of lime pretreatment follow:

1. During pretreatment, the acetyl groups in the xylan polymer are removed resulting in improved cellulase access. [23, 24, 73, 74]
2. Hemicellulose is moderately to well preserved.[11, 75]
3. Delignification is moderate to good.[11, 75]

Alkaline pretreatment of lignocellulosic biomass has had its most long-standing and extensive application in kraft and bleaching processes, which are amply discussed in literature.[76] This pretreatment has also been widely studied as a method to enhance digestibility of crop residues to be used as animal feed. Lime is the most suitable alkali for this application because it is not toxic, is inexpensive, and the pretreatment conditions can be very mild (ambient temperature with pretreatment times ranging from 24 h to several months); thus, a sophisticated or expensive reactor is avoided. This methodology has resulted in moderate to good increase of *in vitro* digestibility as shown in a summary of previous results by Chang et al.[77] In other studies,[78] the *in situ* digestibility (tested in the rumen of a cannulated steer) of bagasse, bajra, jowar, and tobacco stalks was doubled due to lime pretreatment. The pretreatment conditions were lime loading 0.1 g/g dry biomass in boiling water for 1 to 2 h. Further research has shown that lime pretreatment effectively hydrolyzes protein from animal waste (such as

chicken feathers, animal hair or shrimp heads),[79] which results in an additional advantage in animal feed applications.

Another use of alkaline pretreatment is to improve digestibility of lignocellulosic resources to be used in fermentation processes. For example, up to about 170% improvement in methane yield has been observed after applying lime pretreatment to municipal solid waste feedstocks[80] or waste activated sludge.[81] In other instances, the digestibility of multiple lignocellulosic feedstocks (e.g., rice straw,[82] municipal solid waste with sewage sludge,[83] sugarcane bagasse, and corn stover[84]) has been importantly enhanced through lime pretreatment of lignocellulosic feedstocks at temperatures from 50°C to 121°C for several weeks to several hours. In these studies, the digestibility was tested by carboxylic acids yields, which were the expected product.

Finally, alkaline pretreatment has been applied to processes that use the cellulose and hemicellulose polymers from lignocellulosic resources to obtain sugar monomers that are intended for conversion into biofuels (as in Figure 6b). For example, Pan et al. mixed NaOH with steam-exploded Douglas fir wood at 110°C for 3 h, and obtained an approximate lignin removal of 84% (initial content >40%). The remaining cellulose was enzymatically digestible up to 100%.[73] An important drawback to the use of high-temperature NaOH is high degradation of cellulose, which is not observed under gentler alkaline conditions using $Ca(OH)_2$. It has been experimentally demonstrated that carbon dioxide resulting from carbohydrate degradation reacts with calcium hydroxide to form calcium carbonate protective layers that deposit over cellulose preventing large degradation.[85] Consequently, lime pretreatment shows one of the highest glucan

recoveries (>99%[86]). This observation is further supported by the studies performed by Rabelo et al.,[87] who concluded that lime gives better sugar yields than hydrogen peroxide under comparative pretreatment conditions.

Alkaline pretreatment at freezing temperatures has also been tested as reported by Zhao et al.[88] They found that the enzymatic hydrolysis efficiency of spruce was remarkably enhanced (over 60% glucose conversion) by alkaline treatment using 3% NaOH and 12% urea at −15°C for 24 h. No previous results were found testing lime pretreatment at freezing temperatures,

Selected results from systematic research on lime pretreatment are summarized in Table 3. These studies were aimed to identify best pretreatment conditions for different feedstocks based on sugar yields after the combined operations of pretreatment and enzymatic hydrolysis. They used uniform analytical procedures and enzymatic hydrolysis conditions. (Note: Exceptions are the studies from Saha[70, 89] and Rabelo,[87] which are included here only to show a new, increasing interest in lime pretreatment). Overall, notable total sugar yields (>64%, most preferably between 70 and 88%) were obtained. These yields translate into improved biomass digestibility ranging from two to nine times the digestibility of raw biomass; thus, lime pretreatment has proved highly competitive. Additionally, in accord with previous results, glucan recovery after pretreatment proved remarkably high (>90% in all reported cases).

Lime pretreatment is effective across a wide range of temperatures, pressures, and pretreatment times. Furthermore, important improvements were observed for many lignocellulosic feedstocks showing the robustness and effectiveness of the method; thus,

if the appropriate pretreatment conditions are selected, lime pretreatment can be successfully applied to multiple lignocellulosic feedstocks.

Because there is sufficient data, the following generalizations regarding selection of pretreatment conditions can be made based on lignin content and type of feedstocks: Lignin content ≥25% and woody biomass require severe conditions (e.g., 150°C, 200 psig, O_2, 6 h); lignin between 18% and 25% in herbaceous biomass requires mild or moderate conditions (e.g., 55-65°C, 4 weeks with air at atmospheric pressure; or 100 to 120°C without oxygen for 1 to 5 h). These guidelines should be used with caution; the best pretreatment performance is only achieved when the actual temperature, pressure, oxidative agent, and pretreatment time are chosen on individual basis for each biomass, otherwise significant decreases in yields may result. Good examples of this are provided for the cases of corn stover and bagasse. For the former, much better yield was obtained with pretreatment at 55°C, 4 weeks, air than if pretreated at 2 h, 120°C, and without an oxidative agent. In contrast, bagasse showed the completely opposite result. It became more digestible with the shorter pretreatment of only 1 h at 120°C and without air/O_2 than with the longer pretreatment of 4 weeks, air, and 57°C.

Lime pretreatment has proven effective at a wide range of conditions. Broadly, it can be divided into two main modes for studying purposes: (1) Long-term pretreatment, at temperatures up to 75°C, with or without air as oxidative agent. This pretreatment lasts several weeks depending on the desired delignification and the initial amount of lignin in the feedstock. It may be subdivided into oxidative and non-oxidative.

Table 3. Literature review assessing lime pretreatment on the basis of enzyme digestibility.

Feedstock	Lignin (%)	Pret. Time	Oxidative agent	Temp. (°C)	Lime (g/g)	Digest. Increase	TSY %	PGR %	Cellulase Loading	Hydr. Time(h)	Study
Sugarcane	25.8	36 h	None	70	0.40(a)	NR	70.7(d)	NR	3.5(i)	Max(n)	Rabelo, 2008[87]
	21.9	4 weeks	Air	57	0.12(b)	NR	64.3(e)	NR	5.0(i)	72	Granda, 2004[90]
Switchgrass	22.0	1 h	None	120	0.10(b)	4.7×	70.0(f)	93.6	5.0(i)	72	Chang, 1998[91]
	21.7	2 h	None	100	0.10(a)	7×	58.1(f)	90.3	5.0(i)	72	Chang, 1997[92]
Corn stover	20.8	4 weeks	Air	55	0.07(b)	4×	88.1(f)	97.8	15.0(j)	72	Kim, 2005[75]
	21.5	5 h	None	120	0.08(b)	NR	53.3(f)	93.3	10.0(i)	72	Kaar, 2000[93]
	21.5	5 h	None	120	0.10(b)	NR	75.0(f)	93.3	5.0(k)	72	Kaar, 1998[94]
Newspaper N.O.	NR	3 h	7.1 bar O_2	140	0.30(a)	2.2×	62.7(g)	NR	5.0(l)	72	Chang, 2001[86]
	NR	3 h	None	120	0.30(b)	1.7×	49.0(g)	NR	5.0(l)	72	Chang, 2001[86]
Poplar wood	28.0	6 h	14.8 bar O_2	150	0.10(a)	9.1×	77.0(f)	98.2	5.0(l)	72	Chang, 2001[86]
	28.0	30 min	None	240	0.10(a)	7.3×	43.8(g)	NR	5.0(l)	72	Chang, 2001[86]
Wheat straw	NR	24 h	None	50	0.10(a)	8.7×	83.0(g)	NR	5.0(i)	72	Chang, 1998[91]
	NR	1 h	None	121	0.10(a)	2.4×	82.0(f)	NR	0.15(m)	72	Saha, 2007[89]
Rice hulls	15.4(c)	1 h	None	121	0.10(a)	1.7×	32.0(f)	NR	0.15(m)	72	Saha, 2008

N.O: These pretreatment conditions are not optimized. They are the result of exploratory studies only.

(a) Lime loaded.

(b) Lime consumed

(c) Lignin content measured through Van Soest Method. All other lignin contents were measured through H_2SO_4 two stage hydrolysis method. Van Soest Method tends to give lower results.

(d) Measured through DNS method (Total Reducing Sugars, TRS).

(d) Klason and soluble lignin were determined through H_2SO_4 hydrolysis. Ash was determined gravimetrically. Hollocellulose (glucose+xylose) was considered as total mass minus (lignin+ash).

(e) Sugars measured through HPLC

(f) Estimated using total reducing sugars data reported in the paper

(g) Enzymatic hydrolysis time in hours

(h) FPU/g dry treated biomass

(i) FPU/g glucan. For this particular study, this translates to 5.4 FPU/g dry raw biomass

(j) FPU/g dry treated biomass with addition of tween 20 during enzymatic hydrolysis

(k) FPU/g dry raw biomass

(l) mL/g dry treated biomass for each of 3 different enzyme cocktails with varying cellulase activity (celluclast, novozyme and viscostar 150).

(m) Max: Hydrolysis time necessary to obtain no further changes in carbohydrates concentration.

TSY: Total sugar yield. PGR: Pretreatment Glucan Recovery: Percentage of glucan recovered after pretreatment only.

(2) Short-term pretreatment, which is more effective with oxidative agents (oxygen is chosen because it is cheaper than other options), uses temperatures up to 180°C and lasts several hours. The oxygen may be applied in either of two modes: constant pressure and varying pressure.

Reactions during lime pretreatment

In lime (alkaline) pretreatment of lignocellulose materials, the lignin structure is modified because the hydroxyl groups cleave the lignin polymer, which results in partial solubilization.[21, 22] In pulping processes, most lignin degradation mechanisms are known, exhibit strong dependency on temperature and time, and are significantly enhanced by the presence of an oxidative agent.[13, 95-97] Simultaneously, some alkaline degradation of both hemicellulose and cellulose occurs. These reactions, which consume alkali, are mainly cleavage reactions of ether bonds in lignin units and peeling reactions of carbohydrates.[98] Figure 9 illustrates two simultaneous and competitive reactions of the phenolic structures. In both cases an intermediate quinone methide is formed. Delignification and sugar degradation reactions are briefly discussed next.

Alkaline delignification reactions. Alkaline depolymerization of lignin mostly depends on the cleavage of two types of aryl ether bonds: C_{aliph}–O–C_{arom} and C_{arom}–O–C_{arom} (ordered from least to most stable), which frequently correspond to α- and β-aryl ether bonds (50-70% in wood). Examples of these typical delignification reactions (only OH^- anions involved) are presented in Figure 9.

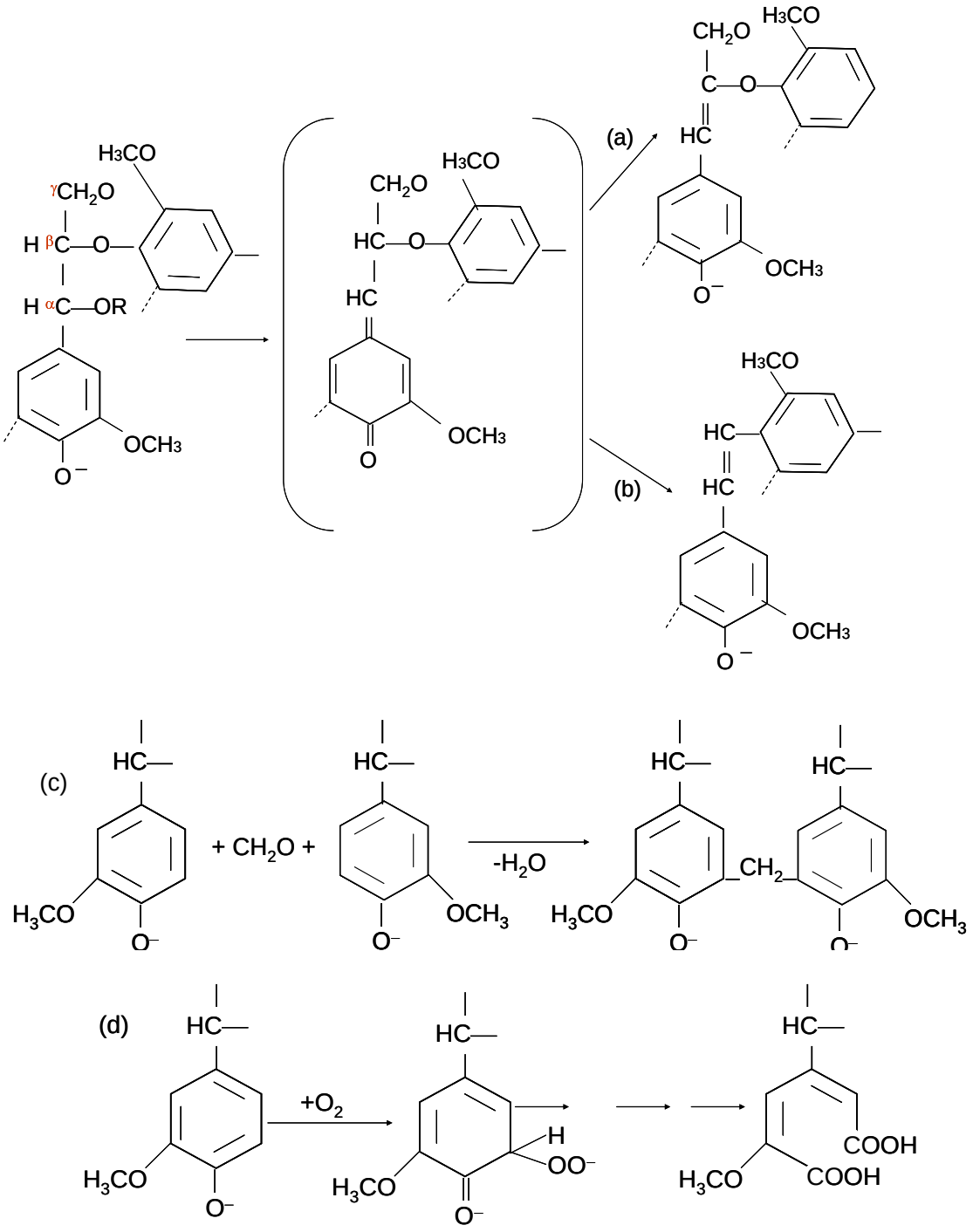


Figure 9. Lignin degradation reactions in alkaline conditions involving α and β-aryl ether linkages[20, 99, 100] (a) cleavage of α-aryl ether linkage (b) cleavage of CH_2O group (c) example of a possible condensation reaction. (d) Example of alkaline oxygen degradation of lignin.

If HS^- were present, reaction (b) would have cleave the β-ether bond instead of the illustrated product; thus, the presence of hydrosulfide anions greatly facilitate delignification because of their greater nucleophilicity.[20] The carbon-to-carbon bonds, especially $C_{arom}–C_{arom}$ are essentially stable.[22]

Because lignin fractions contain reactive groups, undesirable condensation reactions may occur between lignin entities retarding delignification. This is known to occur mostly in terminal phases of delignification processes and at the unoccupied C-5 position of phenolic units. An example of such reactions is illustrated in Figure 9c.

Alkaline oxygen delignification has been extensively studied for bleaching purposes. Oxygen is relatively unreactive and needs free radicals such as transition metal compounds, particularly iron, manganese, and copper (catalyzed oxygen delignification). Alternatively, oxidative lignin reactions are initiated when an ionized phenolic hydroxyl group reacts with oxygen. The production of these phenoxide groups requires very basic conditions (pH >12). Hydroperoxide may also be produced, which depends on pH. This product can participate in further reactions with both lignin and carbohydrates.[101] The reactions involved in alkaline oxidative pretreatments are primarily single-electron (radical) reactions. Oxygen opens rings and cleaves side chains giving a complex mixture of small oxygenated molecules[100, 102, 103] (Figure 9d).Sugar degradation reactions. Although the radical reactions are largely responsible for delignification, they also damage cellulose. Oxygen-based radicals, especially hydroxyl radicals (HO•) can oxidize hydroxyl groups in the cellulose to form ketones. Under the strongly basic conditions used in oxygen delignification, these compounds undergo reverse aldol

reactions that cleave cellulose chains. An example of cellulose breakage is illustrated in Figure 10. An hydroxyl group in one of the cellulose rings is oxidized and the β-linkage is broken at that point by a β-elimination reaction.

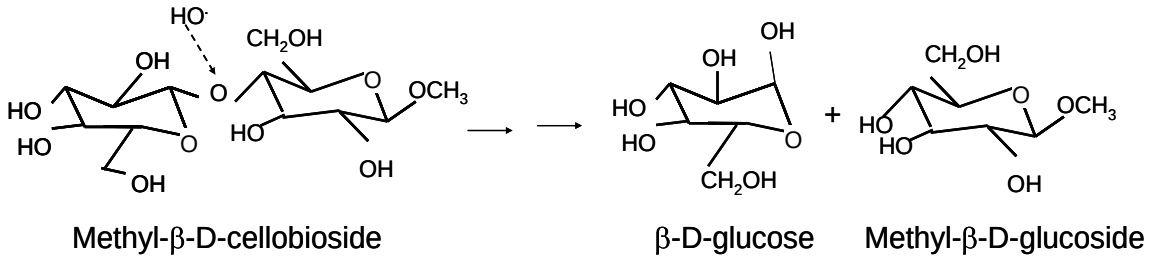


Figure 10. Example of a β-elimination reaction.[104]

Additionally, during kraft pulping, the side group in the xylan backbone, 4-O-methyl-D-glucuronic acid, is partly converted to hexenuronic acid. Simultaneously, degradation reactions of these side groups occur (Figure 11).

In alkaline media, another type of reaction contributing to carbohydrate yield loss in alkaline media is the peeling reaction, in which the cellulose chain is progressively shortened by the loss of single glucose units from one end of the chain. Because oxygen itself converts reducing end groups to the stable oxidized form, peeling reactions are not a major concern in oxygen delignification processes.

The rate constant of the formation of hexenuronic acids through the elimination of methanol from methylglucuronic acids is referred to as k_1. The degradation of eGlcA and HexA is controlled by rate constants k_2 and k_3, respectively.

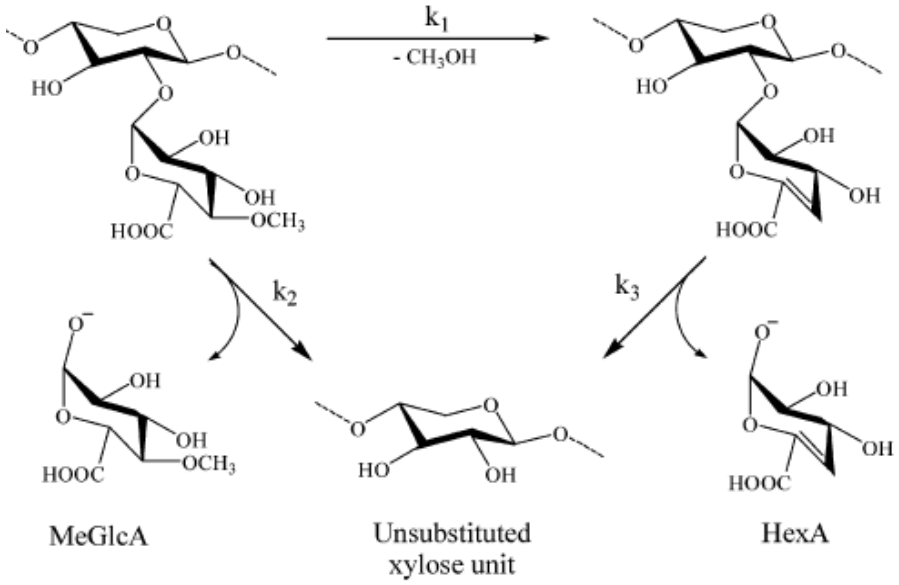


Figure 11. Example of carbohydrate degradation in alkaline oxidation processes.[105]

peeling reaction is similar to that of random chain cleavage because both represent chain breakage at a weak point, the weakness being associated with the presence of a carbonyl group. In random cleavage, the carbonyl group is introduced by oxidation, whereas in peeling it is already there as the terminal aldehyde group at the reducing end of the molecule. In oxygen delignification, magnesium salts are added to help preserve cellulose chains, but mechanism of this protection has not been confirmed.

Acid formation is discussed separately because lignin, cellulose, and hemicellulose are parent molecules. Produced acids may consume alkali and lower the pH, or can lead to sugar degradation. In lignin, conjugated acids may arise in oxidative alkaline treatment by cleaving the Cα–Cβ bond of etherified structures containing α-carbonyl group. This reaction produces benzylic-type carboxyl acids. As discussed by

Gierer,[106] the carbonyl moieties are readily attacked by oxygen. The oxidative structure produces a four-membered oxirane intermediate that can undergo a ring opening or yield conjugated aromatic acids (Figure 12).

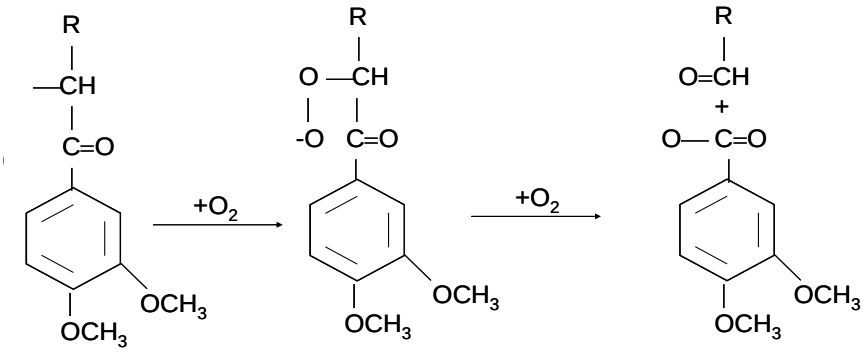


Figure.12 Example of a lignin reaction that lead to acid production.[103]

On the other hand, in alkaline solutions, glucose is present in its enolate form, which is oxidized via a one-electron transfer to molecular oxygen to give carbonyl structures (glucosones) and further oxidation products (Figure 13). The formation of carbonyl structures in cellulose may lead to alkali-induced cleavage of glucosidic bonds (peeling).

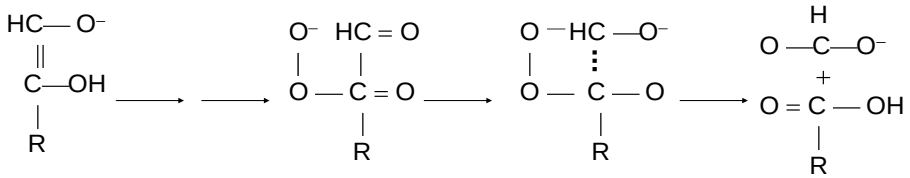


Figure 13. Example of a glucose reaction that lead to acid production.[100]

LONG-TERM LIME PRETREATMENT OF POPLAR WOOD*

Synopsis

Long-term lime pretreatment has proven to increase digestibility of many herbaceous lignocellulose sources; but until this work, its effects had not been evaluated on wood, whose lignin content is higher and therefore more recalcitrant to enzymatic hydrolysis. In this study, the mild conditions of long-term lime pretreatment (1-atm pressure, temperatures ranging from 25 to 75°C, and reaction times between 1 and 12 weeks, with and without air) were systematically applied to poplar wood available in two batches with different lignin contents. These batches were designated as Low Lignin Biomass (LLB) with lignin content of 21.4% and High Lignin Biomass (HLB) with lignin content of 29.1%. Full factorial designs resulted in 79 samples of pretreated poplar that were analyzed for lignin and carbohydrates pretreatment yields, and enzymatic digestibility (15 FPU/ g glucan in raw biomass cellulose loading). After aerated lime pretreatment at 65° for 4 weeks, and subsequent enzymatic hydrolysis, an overall yield of 0.76 g glucan+xylan recovered/g glucan+xylan in raw biomass was obtained. This is equivalent to an increased poplar wood digestibility of 7.5 fold compared to untreated biomass. Different batches of the feedstock resulted in different

*Reprinted with permission from Long-term Lime Pretreatment of Poplar Wood by Sierra, R., Holtzapple, M., and Granda, C. AIChE Journal. In Press. Copyright (2010) by Wiley and Sons.

lignin and carbohydrates pretreatment yields; however, overall yields of carbohydrates (combining pretreatment and enzymatic hydrolysis) were similar.

Introduction

Long-term lime pretreatment is initiated by mixing lignocellulosic biomass with excess calcium hydroxide (lime loaded as 0.5 g/g dry biomass) and water (9 to 15 g/g dry biomass). Then, for days to weeks, the mixture is exposed to temperatures ranging from 25 to 75°C at atmospheric pressure, preferably with aeration.[107] This procedure significantly increases lignocellulosic biomass digestibility making it useful as livestock feed or feedstock for fermentation processes to produce liquid fuels or chemicals.[108]

Chemically, the main results of alkaline pretreatments are cleavage of lignin polymer and hydrolysis of acetyl groups on hemicellulose; however, some undesirable carbohydrate degradation also occurs.[91 75] These outcomes result from reactions between hydroxyl groups and lignocellulose through mechanisms that strongly depend on temperature and time. Among alkalis, lime is preferred because it is safe to handle, inexpensive, easy to recover, compatible with oxidants, and results in good carbohydrate preservation.[85] Under alkaline conditions, lignin removal is significantly enhanced by oxidative agents.[95-97] Among these, air is preferred because it is inexpensive.

A previous study with herbaceous biomass showed that wheat straw (lignin content not reported) and sugarcane bagasse (22% lignin) treated with lime without oxidant for 24 hours at 65°C increased digestibility 3 to 4 times compared to raw biomass.[91] In another study, sugarcane bagasse was lime treated with air for 4 weeks at

57°C which resulted in an increased sugar yield of about 5 times compared to raw biomass.[90]Corn stover (18% lignin) treated with lime and air for 4 weeks at 55°C gave overall yields of glucose and xylose of 93.2% and 79.5%, respectively.[75]

Compared to herbaceous biomass, wood is more recalcitrant because of higher lignin content;[109] however, even in this case, alkali pretreatment increases biomass digestibility. For example, aspen soaked in NaOH solution at room temperature for 1 hour increased digestibility (32%) with similar results for black ash (17%) and soft maple (20%).[110]

This work assesses long-term lime pretreatment of poplar wood on the basis of sugar yields using two different batches with different lignin contents (21.4% and 29.1%). The resulting mass balances, pretreatment yields, and overall yields are reported after pretreatment, enzymatic hydrolysis and the combined pretreatment and enzymatic hydrolysis. This article statistically compares pretreatment yields, enzymatic digestibility of pretreated samples against untreated samples, the two biomass batches, and the effects of the presence or not of air during pretreatment.

In a previous study, lime pretreatment was successfully applied to poplar wood (HLB) at temperatures ranging between 110 and 180°C, oxygen pressure ranging between 14.8 and 28.1 bar, and pretreatment times between 1 and 6 hours.[111] This type of lime pretreatment (designated as short-term pretreatment) is compared to long-term pretreatment for poplar wood elsewhere.[112]

Materials and methods

Feedstock. Hybrid poplar wood (var NM6, genotype *P. nigra* × *P. maximowiczii*), was kindly provided by the National Renewable Energy Laboratory (NREL) in two batches. The procedure to prepare the poplar wood and reduce its particle size is explained elsewhere.[12] For low-lignin biomass (LLB), the composition of raw poplar wood determined by NREL is 45.1% glucan, 17.8% xylan, 1.7% mannan, 21.4% lignin, 1.5% galactan, 0.6% arabinan, 3.4% extractives, 0.8% ash, and 5.6% acetyl. For high-lignin biomass (HLB), the composition is 43.8% glucan, 14.9% xylan, 3.9% mannan, 29.1% lignin, 1.3% galactan, 0.7% arabinan, 3.3% extractives, 1.1% ash, and 3.3% acetyl.

Pretreatment. Lime pretreatment was performed in packed-bed reactors made of PVC pipe (1-inch = 0.0254-m ID, 17 inch = 0.432-m length) jacketed with larger diameter PVC pipes (2-inch = 0.0508-m ID, 15-inch = 0.381-m length). The desired temperature was maintained by constantly pumping water (3/4-hp = 0.560-kW centrifugal pumps, TEEL, Niles, IL) through the jacket from tanks (8-gallon = 0.0302-m^3, Nagalene Co, Mickleton, NJ) equipped with heating elements, a temperature controller (Omega Engineering, Inc., Stamford, CT), and a liquid-level controller (McMaster-Carr, Inc. Atlanta, GA). Several temperatures and aeration conditions were run simultaneously by using 40 of these PVC reactors attached to a metal frame, four pumps, and four tanks (Figure 14). Once the pretreatment temperature was reached, biomass (15 g dry weight), excess lime (7.5 g calcium hydroxide, certified), and distilled

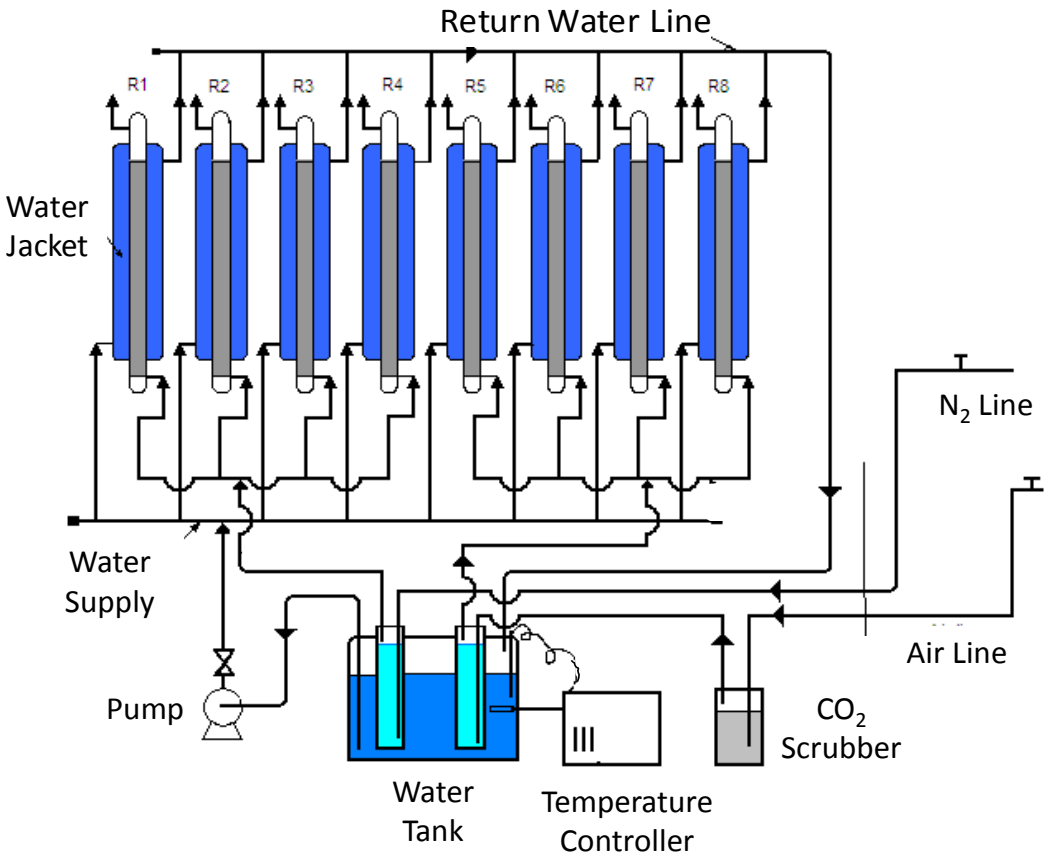


Figure 14. Schematic of a set of eight reactors used for pretreatment.

water (150 g) were mixed and charged into the reactors, thereby occupying 79% of reactor total volume. pH of the samples obtained in this way ranged between 11 and 11.5.

For some pretreatments, aeration was provided in gross excess using compressed air that was continuously bubbled into the reactors from the bottom at a flow rate of about 3.5 mL/min. (air residence time 62 min and air superficial velocity 6.91 mm/min). This air flow rate was controlled by clamps located at the inlet. Before entering the

reactors, the air was scrubbed of carbon dioxide by passing through a mixture of lime and water in order to limit neutralization of the lime in the reactors by the carbon dioxide in the air. After this, air was preheated and humidified by passing through a cylinder containing water at the pretreatment temperature.

Aeration was then started by bubbling compressed air into reactors using valves and clamps to control flow rate. To stop pretreatment, the air valves were closed, the pumps were turned off, the reactors were removed from the metal frame, and their content was carefully and completely transferred to properly labeled 1-L centrifuge bottles using approximately 500 mL of clear distilled water. pH of this mixture ranged between 10 and 11.

Lime consumption and biomass conditioning. To calculate unreacted lime, the biomass and liquid mixture obtained after pretreatment were carefully titrated using 5-N HCl. During pretreatment, lime was consumed in the following reactions: (1) neutralization of acetic acid (coming from acetyl in biomass), (2) neutralization of minerals in ash, (3) reactions that degrade/solubilize lignin and carbohydrates.

Lime consumed in reactions (1) and (2) was low and uniform for all pretreated samples because ash and acetyl contents are low (see Section *Feedstock*). Furthermore, acetic acid in pretreated samples was <0.01% regardless pretreatment conditions (measured using NREL Standard Analytical Procedure[113]); thus, lime consumption on reactions (1) and (2) was low and uniform for all samples. Consequently, differences in

lime consumption for different pretreatment conditions are always due to reactions between the highly alkaline-oxidizing reaction media and lignin and carbohydrates.

After neutralization, the samples were extensively washed with deionized water and filtered using a vacuum filtration apparatus with Whatman 934/AH glass fiber filter paper (particle retention = 1.5 μm, Fisher Scientific Co., Pittsburgh, PA). Subsequently, the biomass was air dried. The weight of dried biomass and its moisture content were recorded to account for pretreatment yield of solids (undissolved biomass).

Pretreatments for HLB were run using a full factorial experimental design with the following factors and levels: temperature (25, 35, 45, 55, and 65°C), time (1, 2, 4, 7, 8, and 12 weeks), and aeration (level 1 with air and level 2 without air), generating a total of 60 pretreated samples plus a replicate for each. All pretreatments for LLB were run with aeration and used a full factorial experimental design with the following factors and levels: temperature (55, 65, and 75°C) and time (1, 2, 3, 4, and 8 weeks) obtaining a total of 15 samples plus a replicate for each. To meaningfully compare HLB yields to LLB yields, HLB was also submitted to aerated pretreatments at 75°C for 1, 2, 4, and 8 weeks. Data for this higher temperature are shown in Table 4. After pretreatment, all samples were assessed for compositional analysis and enzymatic digestibility using the following laboratory analytical procedures.

Table 4. Pretreatment and overall yields for HLB and LLB at 75°C.

Time (weeks)	Pretreatment Yields[a]						Overall carbohydrates combined yields[b]	
	Lignin		Glucan		Xylan			
	HLB	LLB	HLB	LLB	HLB	LLB	HLB	LLB
0	1.00	1.00	1.00	1.00	1.00	1.00	0.10	0.09
1	0.76	0.75	0.93	0.87	0.98	0.80	0.33	0.41
2	0.61	0.65	0.93	0.85	0.85	0.65	0.41	0.50
4	0.50	0.56	0.79	0.69	0.74	0.55	0.58	0.49
8	0.31	0.35	0.65	0.48	0.69	0.34	0.55	0.46

[a] g component/g component in raw biomass
[b] g glucan+xylan/g glucan+xylan in raw biomass

Biomass composition. Analysis of raw and pretreated poplar wood was performed on samples with a particle size between 20 mesh (0.850 mm) and 80 mesh (0.180 mm), and a moisture content ≤10% as suggested by NREL Standard Analytical Procedures.[114] Extractives (i.e., chlorophyll, waxes, or similar organic components) were separated using 95% ethanol in an exhaustive extraction performed in a Soxhlet apparatus for 24 h. After extraction, the solvent was removed using a rotary evaporator (Buchi, Model 121), yielding the extracted compound that was quantified gravimetrically.[115] Carbohydrate, lignin, and acetic acid content were determined by submitting extractives-free samples to two-stage acid hydrolysis procedure.[113] The analyses for carbohydrates and acetic acid were performed on the resulting hydrolyzate by HPLC with refractive index detection using Biorad HPX-87P and HPX-87H columns, respectively. The lignin content was determined gravimetrically as the weight of solids after acid hydrolysis, discounting moisture and ash. Ashing was performed at 575 ± 25°C.[116]

Pretreatment liquor. The pretreatment liquor was separated from the solids through vacuum filtration. The concentrations of soluble monosaccharides and cellobiose were determined using HPLC equipped with Biorad HPX-87P and HPX-87H columns, and with refractive index detection. Whenever cellobiose was detected, the concentration was converted to glucose concentration using the conversion factor suggested in Section 8.20 of NREL Analytical Laboratory Procedure.[117] Oligomers were hydrolyzed before HPLC measurements by submitting the pretreatment liquor to acid hydrolysis (4% H_2SO_4) and then measuring dissolved sugars using HPLC with a Biorad HPX-87P column.[118]

Enzymatic hydrolysis. The sole criterion to determine recommended pretreatment conditions was the combined glucan and xylan yields after pretreatment and enzymatic hydrolysis. The cellulase (Spezyme CP®, lot 301-04075-054, activity 59 FPU/mL) used in this study was kindly provided by Genencor International, Inc. Its activity was monitored on a regular basis using NREL Standard Analytical Procedure.[119]A cellulase enzyme loading of 15 FPU/g glucan in raw biomass was used. β-glucosidase (Novozyme 188®, 288 CBU/g of activity as measured by Novo Nordisk Biochem) from Sigma-Aldrich was added with an excess loading of 60 CBU/g glucan in raw biomass.

The substrates used in this study were raw poplar wood and treated-neutralized-washed poplar wood. Based on moisture content, glucan content, and the solids pretreatment yield (dry weight pretreated biomass per weight of dry raw biomass), enough substrate was weighed to provide 0.1 g glucan for the reaction. Water, sodium

citrate buffer (0.1 M, pH 4.8), antibiotics (tetracycline, 10 mg/mL in 70% ethanol and cycloheximine, 10 mg/mL in distilled water), and enzymes were added to the substrate to bring the total volume of the mixture to 10 mL.[120] After 72 hours of hydrolysis at 50°C in a shaking incubator (Amerex Instruments Inc, Laffayette, CA, 80 rpm), the sugar yields were measured by HPLC using Biorad HPX-87P column with refractive index detection.

Data analysis method. Assessment of pretreatment yields of lignin, glucan, and xylan was based on the following definition for yield:

$$Y_i = \frac{C_i Y_T}{C_{i_0}} \tag{1}$$

where

i = lignin L, glucan G, or xylan X

Y_i = pretreatment yield of Component i at time t (kg residual Component i/kg Component i in raw biomass)

C_{i0} = Component i content at time zero (kg Component i in raw biomass/kg raw biomass)

C_i = Component i content at time t (kg residual Component i/kg residual biomass)

Y_T = total solids pretreatment yield at time t (kg residual biomass/kg raw biomass)

The highest possible yields are 1.0 and the amount of degraded component is 1.0 – Y_i. The effects of pretreatment on lignin, glucan, and xylan yields for HLB are

discussed in the next section and the differences with LLB are addressed in Section *Comparing HLB and LLB pretreatment yields*.

Overall yield (Y_{oi}) is defined as the amount of glucan or xylan recovered after pretreatment and enzymatic hydrolysis per unit of cellulose or hemicellullose in the raw feedstock. Y_{oi} and was calculated as:

$$Y_{oi} = Y_i \cdot Y_{ei} \tag{2}$$

where

i = Glucan G or xylan X

Y_{oi} = overall yield of Component i (kg hydrolyzed Component i/kg Component i in raw biomass)

Y_i = pretreatment yield of Component i (kg residual Component i/kg Component i in raw biomass)

Y_{ei} = enzymatic yield of Component i (kg hydrolyzed Component i/kg Component i in pretreated-neutralized-washed biomass)

To statistically determine significant differences of interest, Student t-tests and Analysis of Variance (Anova) were performed using Minitab® 15. Complete presentation of program outputs and check of statistical assumptions can be found elsewhere.[121]

Results and discussion

Pretreated solids. Figure 15 shows that lignin, glucan, and xylan yields smoothly decreased with increasing time and temperature. Compared to lignin, the degradation

extent was smaller for xylan and much smaller for glucan. According to Eq. 1, lignin, glucan, and xylan maximum yields were 1.0 and corresponded to untreated samples. The minimum yields were 0.29, 0.60, and 0.38 g component remaining/g component in raw biomass, respectively and corresponded to the aerated pretreatment at 65°C for 12 weeks. These data are important to this study because they indicate overall performance of lime pretreatment on wood. These average yields were 0.76, 0.92, and 0.78 g component remaining/g component in raw biomass, respectively. The average glucan yield is very high showing good cellulose preservation. On the other hand, typical lignin yields were much lower showing that lime pretreatment selectively removes lignin . Covalent bonds between hemicellulose and lignin have been demonstrated for grasses[122] and may be the case for other herbaceous biomass[123, 124] but this type of bonding has not been proved for woody biomass (a good discussion can be found in the review by

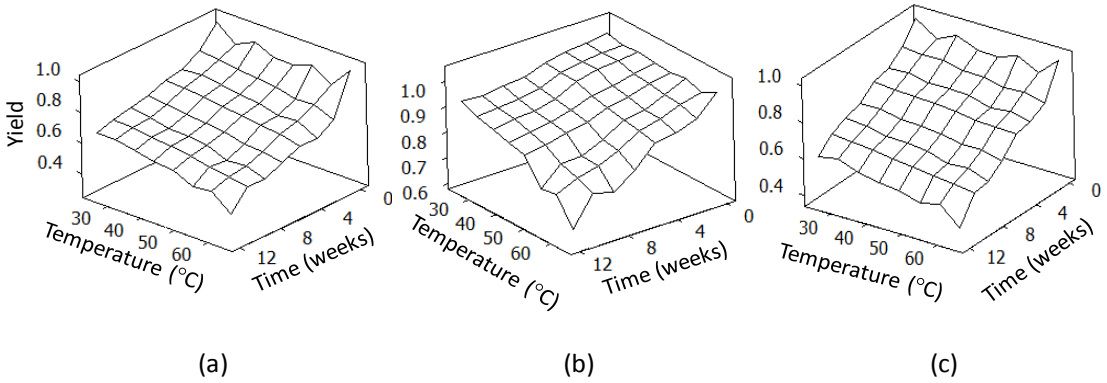


Figure 15. Effects of temperature and time on HLB pretreatment yield. (a) lignin, (b) glucan, and (c) xylan expressed as g of component removed/g component in raw biomass.

Helm.[125]) In any case, lignin and hemicellulose are closely associated with each other, which would explain why lignin and hemicellulose yields are similar.

Figure 16 shows degraded glucan ($1.0 - Y_G$) and degraded xylan ($1.0 - Y_X$) compared against degraded lignin ($1.0 - Y_L$). Glucan and lignin degradations are weakly related (lower slope and lower coefficient of determination), whereas xylan and lignin degradations are strongly related (higher slope and higher coefficient of determination) indicating that lignin is structurally more related to xylan than to glucan. More insights on these phenomena are obtained through kinetic modeling of lime pretreatment, which allows estimation of lignin, glucan, and xylan yields, and also calculation of selectivity as a function of pretreatment conditions.[126]

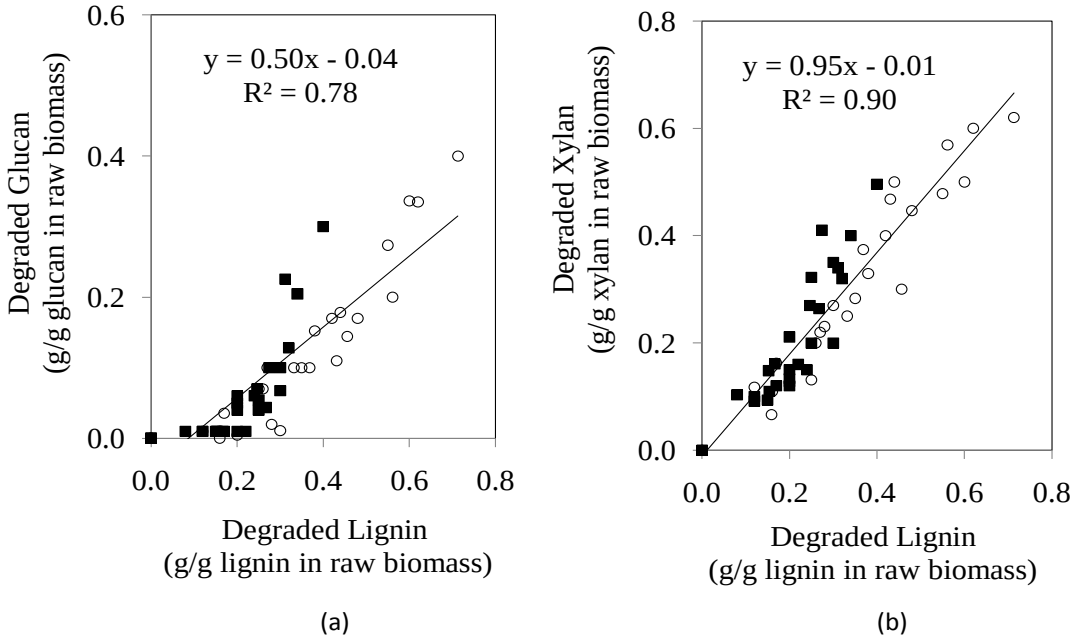


Figure 16. Degraded (a) glucan and (b) xylan compared to degraded lignin for HLB pretreatment ○ aerated ■ non-aerated.

Aerated and non-aerated modes were compared by calculating average differences in yields using a two-sample *t*-test for each of the three components: lignin, glucan, and xylan (the hypotheses were for differences in average yields in the non-aerated mode minus average yields in the aerated mode). The results for lignin, glucan, and xylan with the corresponding confidence intervals were 0.12 ± 0.081, 0.055 ± 0.048, and 0.082 ± 0.087 g remaining component/g component in raw biomass, respectively. In the same order, the *p*-values were 0.005, 0.027, and 0.120. These results indicate that lignin and glucan yields in the aerated mode are significantly different and smaller than the corresponding yields for non-aerated pretreatment (within 5% significance level). For xylan, the high *p*-value shows that there may be an effect due to the presence of air, but it is less notable. This suggests that mechanisms for xylan degradation are not as strongly influenced by oxygen; but it is more likely alkaline-catalyzed hydrolysis than radical attack, unlike lignin degradation.

Regarding other components, total removal of acetyl and partial (50%) removal of extractives were observed within the first week of pretreatment and remained almost constant until the 12^{th} week of pretreatment. Complete mass profiles were obtained for all components at all pretreatment conditions, but the only results presented here are for aerated pretreatments at 65 and 25°C (Figure 17). For both aerated and non-aerated pretreatments, the general tendency is for rapid solubilization of components during the first one to two weeks. Later, solubilization rates were almost constant and generally higher for the aerated mode. These results show that lime pretreatment significantly changes biomass composition, with the strongest effect on lignin removal.

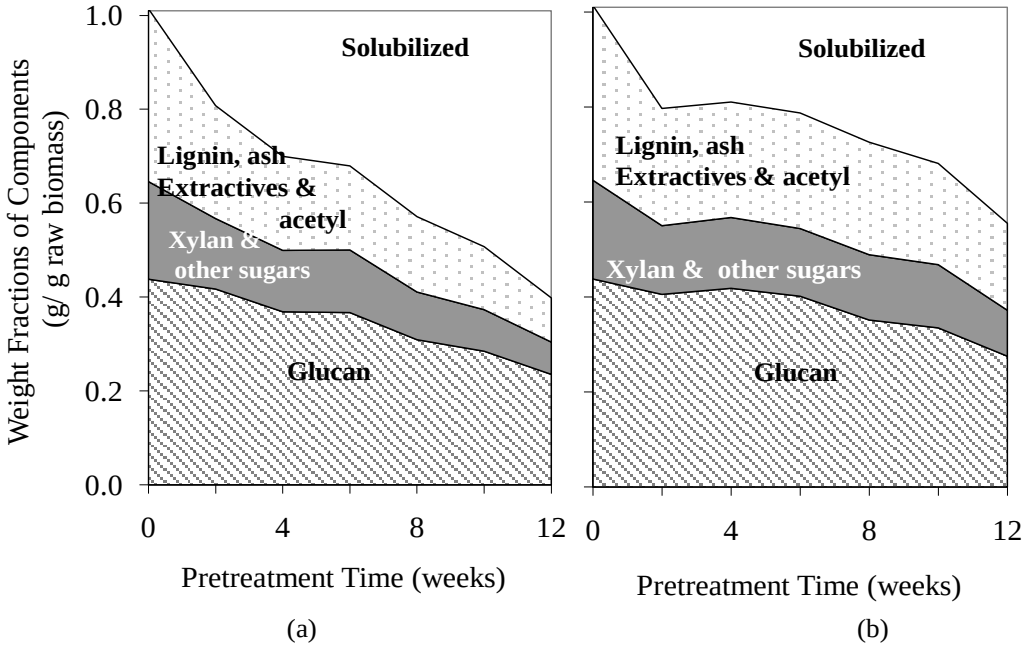


Figure 17. Mass profiles of raw and treated HLB poplar wood with aeration (a) 65ºC and (b) 25ºC.

Comparing HLB and LLB pretreatment yields. Figure 18 compares HLB yields against LLB yields for oxidative pretreatment at 65°C. The significance of observed differences was assessed through an analysis of variance, which showed that temperature, time, and batch have significant effects on lignin and glucan yields at α = 3%; however, xylan yield is not affected by temperature, only by time and batch (the *p*-values were 0.643 for temperature and <0.012 for time and batch).

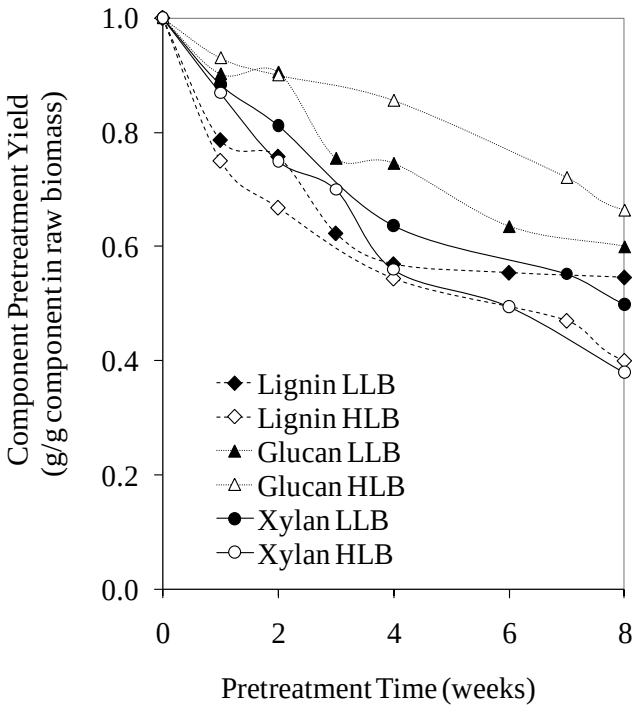


Figure 18. Comparative effects of aerated pretreatment at 65ºC on different batches of poplar wood.

Factors that may be responsible for the differences between the two batches of poplar wood are (1) diverse composition or distribution of lignin and hemicellulose, (2) distinct spatial configurations, (3) dissimilar bonding between lignin units, (4) different bonding between lignin and carbohydrates, and (5) important differences in the initial amount of lignin and carbohydrates for the two batches of biomass. Unfortunately, more specificity regarding possibledifferences is out of the scope of this paper, but it is clear that for HLB, xylan and lignin degradation were higher whereas glucan preserved better.

According to the above referenced Anova, xylan yield is independent of temperature. This result is opposite to the result shown in the previous section for a wider range of temperatures (25 to 65°C), implying that temperature has an effect on

xylan degradation, but the range from 55 to 75°C is too small to be significant. Furthermore, xylan yield was independent of aeration (as discussed in the previous section); thus, unlike lignin and glucan degradation, xylan degradation is not strongly influenced by reaction conditions (temperature, aeration) provided temperature >55°C. Instead, xylan degradation depends primarily on time.

In this comparative study, temperature was increased to 75°C. Inherent delignification rates are triggered by higher temperatures, *i.e.*, rate constants are higher.[126] However, because long-term lime pretreatment uses 1-atm total pressure, higher temperatures increase water vapor pressure and reduce oxygen partial pressure. Table 5 shows that at 75°C, oxygen partial pressure reduces by 36% compared to the partial pressure at 25°C.[11] According to the statistical analysis, a temperature of 75°C significantly affects lignin degradation; however, it also degrades carbohydrates more rapidly. The total effect of this temperature can only be evaluated by calculating overall pretreatment and enzymatic hydrolysis yields (Eq. 2) as discussed in Section *Enzymatic and Overall Hydrolysis Yields*.

Pretreatment liquor. Sugars in the pretreatment liquor were <0.010 g sugar recovered/g sugar in raw biomass. Glucan oligomers were 0.010 g glucan recovered/g raw biomass, and xylan oligomers were 0.013 g xylan recovered/g raw biomass. Even though degradation products from lignin and carbohydrates reactions triggered in the alkaline media are present in the pretreatment liquor, these were not quantified because

previous studies showed that lime pretreatment degradation products do not inhibit fermentation.[127]

Lime consumption. Longer pretreatments, higher temperatures, and aeration cause higher lime consumption (Figure 19). These effects were statistically ascertained through an analysis of variance that gave *p*-values of 0.018 for temperature, and <0.001 for both time and aeration. Thus, the effects of temperature, time, and aeration on lime consumption are statistically significant at α = 2%. The presence of oxygen provides new pathways to degrade lignin, including a dominant phenolic delignification (oxidation of the phenolic subunits of lignin rather than the non-phenolic). In other words, unlike the non-oxidative alkaline process, oxidation attacks C–C bonds.[100]

Table 5. Percentage decrease in oxygen partial pressure in air saturated with water at temperature *T* (ºC) compared to 25ºC[a].

Temperature (ºC)	Saturation Pressure (kPa)	Vapor Pressure (kPa)	Oxygen (molar fraction)	Decrease in oxygen concentration (%)
25	3.17	1.91	0.203	0.0
50	12.4	7.41	0.184	9.35
55	15.8	9.45	0.177	12.8
57	17.9	10.7	0.173	15.0
60	19.9	12.0	0.169	17.1
65	25.0	15.0	0.158	22.3
70	31.2	18.7	0.145	28.5
75	38.6	23.1	0.130	36.1
80	47.4	28.4	0.112	45.1
85	57.8	34.7	0.090	55.7
90	70.1	42.1	0.065	68.2

[a] Saturated air (100% humidity) and standard atmospheric pressure 101,325 Pa.

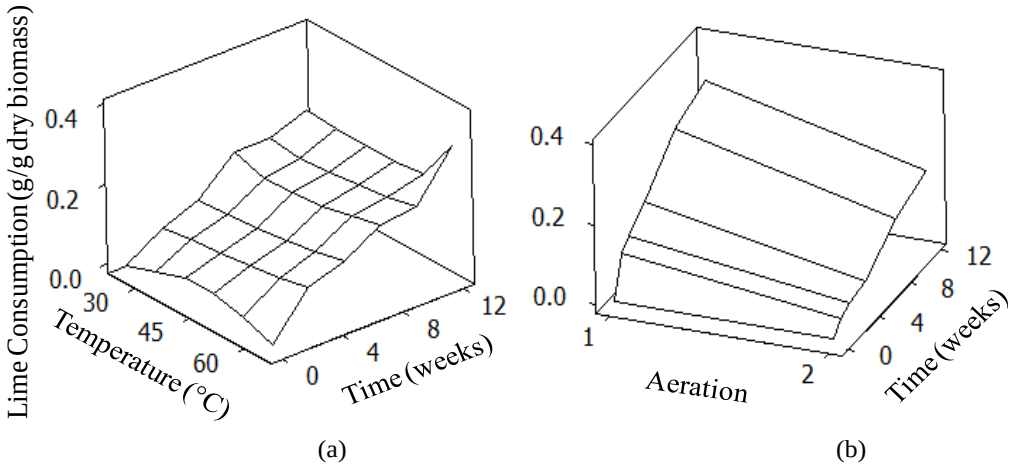


Figure 19. Surface plots to assess the effects of (a) temperature and time averaged over aeration, and (b) aeration and time averaged over temperature on lime consumption during pretreatment of HLB. Lime consumption is expressed as g $Ca(OH)_2$ consumed per g dry biomass.

Average lime consumption was 0.11 g $Ca(OH)_2$/g dry biomass. The maximum was 0.33 g $Ca(OH)_2$/g dry biomass and was observed for 12-week oxidative pretreatment at 65°C.

Lime consumption was 0.20 g $Ca(OH)_2$/g dry biomass (or 40% of initially loaded lime) for the recommended conditions of aerated pretreatment at 65°C for 4 weeks (see Section *Enzymatic and overall hydrolysis yields*). The relationship between lime consumption and lignin or carbohydrate removal is linear (Figure 20), particularly for lignin with aeration. This linearity means that lime is stoichiometrically consumed in the degradation reactions. Table 6 shows parameters for linear regression models for both lignin and carbohydrates in the aerated and non-aerated modes, with their statistical indicators.

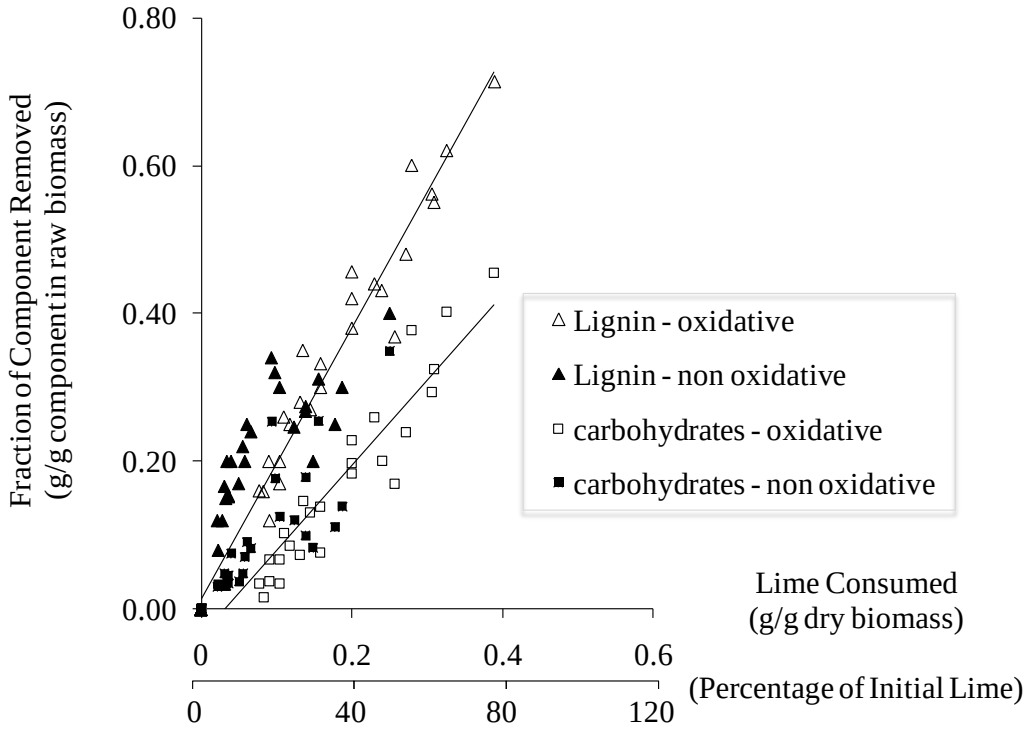


Figure 20. Component removed as a function of lime consumed for HLB.

Table 6. Regression parameters and statistical indicators for a linear regression model relating lime consumption to lignin or carbohydrates degradation.

Regression Model	Intercept		Slope		R-squared	Regression
	a	*p*-value	*b*	*p*-value		*p*-value
$LD^a = a \times LC^b + b$ Aerated	1.83	<0.001	0.015	0.279	95.8	<0.001
$CD^c = a \times LC^b + b$ Aerated	1.14	<0.001	–0.035	0.015	89.8	<0.001
$LD^a = a \times LC^b + b$ Non-aerated	1.40	<0.001	0.085	<0.001	67.5	<0.001
$CD^c = a \times LC^b + b$ Non-aerated	1.08	<0.001	0.0075	0.572	68.6	<0.001

[a] Lignin degraded
[b] Lime consumed
[c] Carbohydrates degraded

Compared to the non-aerated counterpart, the aerated mode shows better fit and higher slopes for both lignin and carbohydrates. Consequently, in the presence of air, lignin and carbohydrate degradation are greater per unit of consumed lime. In other words, the aerated mode consumes less lime to obtain a desired delignification level.

Enzymatic and overall hydrolysis yields. This study was designed to assess pretreatment not to optimize enzymatic hydrolysis variables; thus, fixed and favorable enzymatic hydrolysis conditions were used (see Section *Enzymatic Hydrolysis*). Hemicellulose hydrolysis was not specifically addressed by the addition of xylanases to the enzymes cocktail. Spezyme CP®, which is a *Trichoderma reesei* cellulase enzyme complex, includes some xylanase activity; thus, hemicellulose hydrolysis under the conditions used in this study has been observed by others.[71]

To select the best pretreatment conditions, the sole criterion was the overall combined glucan and xylan yield, which is discussed next for HLB. (Note: Results for LLB are presented in Section *Comparing HLB and LLB Overall Yields*).

Figure 21a shows that glucan and xylan digestibilities of pretreated biomass are closely correlated; as xylan digestibility improves, so does glucan digestibility.[75] Interestingly, Figure 21b shows that as lignin is removed, there is strong preference for glucan hydrolysis over xylan hydrolysis. This result can be explained by the fact that as lignin is removed, hemicellulose is preferentially removed (see Figure 16). As hemicellulose is removed, it opens pores giving greater access to cellulose, similar to what is observed in dilute acid pretreatment.[128]

Furthermore, Figure 21c shows that at 65°C, there is a preference for glucan digestion if the biomass was pretreated with aeration (i.e., ratio is generally >1.0), whereas there is a preference for xylan digestion if the biomass was pretreated without aeration (i.e., ratio is less than 1.0). As pretreatment proceeds, both aerated and non-aerated modes show increasing preference for glucan (i.e., the slopes for both lines are positive). More lignin and hemicellulose removal occurs at longer pretreatments, so there is greater enzymatic access to cellulose, which increases its digestibility.

The maximum glucan digestibility was obtained in the aerated mode for the 65°C pretreatment that lasted 4 weeks. Under these conditions, glucan yield was 0.95 g/g glucan in treated biomass with a corresponding xylan digestibility close to 0.80 g/g

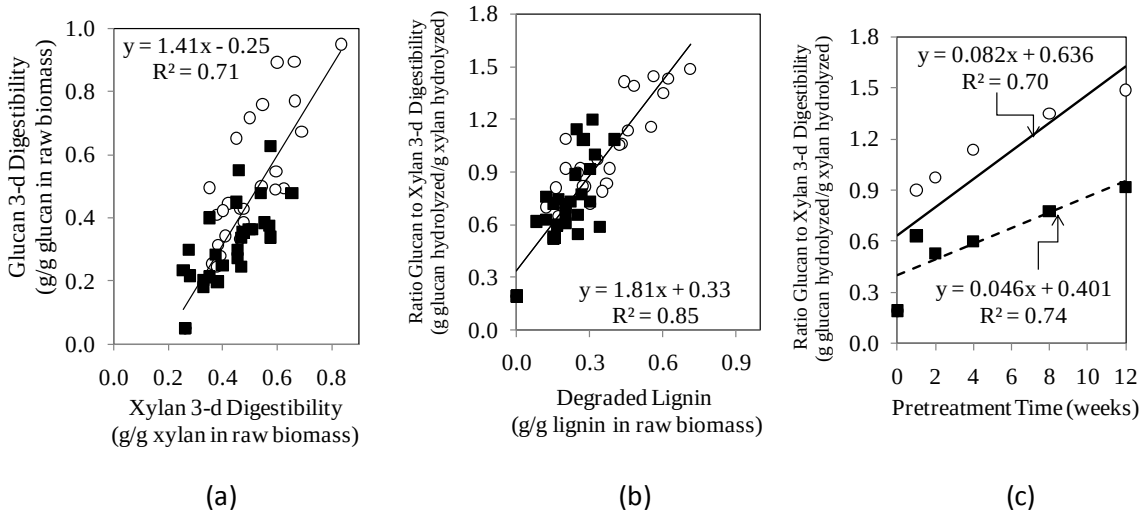


Figure 21. (a) Glucan digestibility compared to xylan digestibility. Ratio of glucan to xylan compared to (b) degraded lignin (c) 65°C pretreatment time. ○ aerated pretreatment ■ non-aerated pretreatment.

xylan in treated biomass. In the non-aerated mode, the maximum glucan and xylan digestibilites were both close to 0.60 g/g carbohydrate in treated biomass.

Figure 22 shows the effect of pretreatment variables on combined glucan and xylan overall yields. Higher temperatures and aeration improve yields showing a slight constant positive slope. Conversely, pretreatment times lower than two weeks gave very low yields. Using Anova, the effect of temperature, time, and aeration on the overall combined yield was corroborated with *p*-values <0.030.

A contour plot (Figure 23) shows that the most promising overall yields are approximately constant for times >2 weeks and temperatures ≥55°C. For these conditions, average overall combined yields were 0.52 and 0.39 g glucan+xylan recovered/g glucan+xylan in raw biomass for the aerated and non-aerated pretreatments,

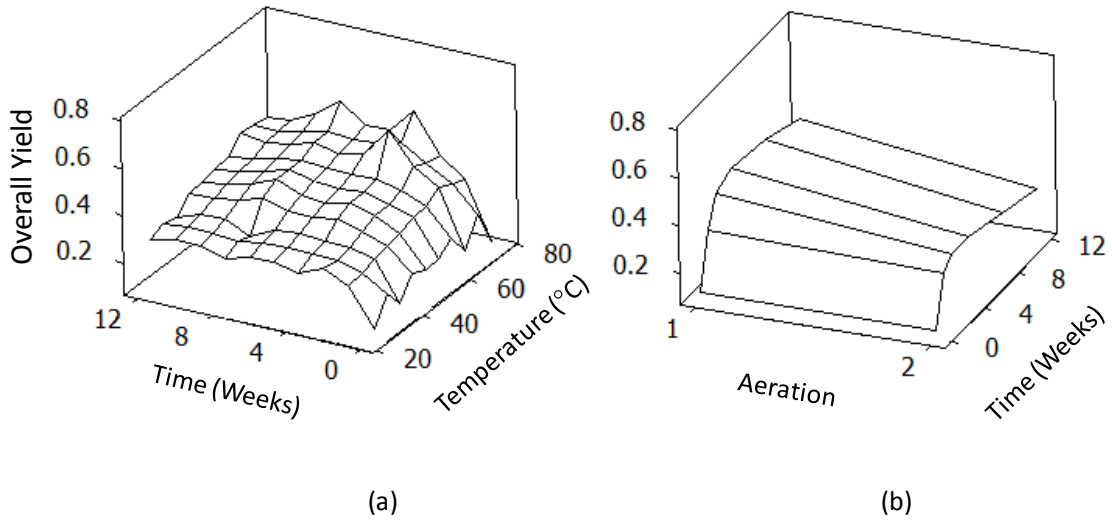


Figure 22. Surface plots to assess the effects of (a) temperature and time averaged over aeration and (b) time and aeration averaged over temperature on overall yield of combined glucan and xylan of HLB expressed as g glucan+xylan recovered per g glucan+xylan in raw biomass.

respectively. Considering the combined overall yield of raw poplar (0.10 g glucan+xylan recovered/g glucan+xylan in raw biomass), this result indicates that aerated lime pretreatment consistently increases the digestibility of raw poplar wood by an average factor of about 5 with respect to raw biomass. The highest improvement in digestibility was about 7.5 fold (0.76 g glucan+xylan recovered/g glucan+xylan in raw biomass) obtained for aerated pretreatment at 65°C and 4 weeks; consequently, these are the recommended pretreatment conditions for poplar wood. More severe oxidative lime pretreatment conditions (oxygen pressure up to 28 bars and temperature up to 180°C) result in a much higher poplar wood digestibility as discussed elsewhere.[111]

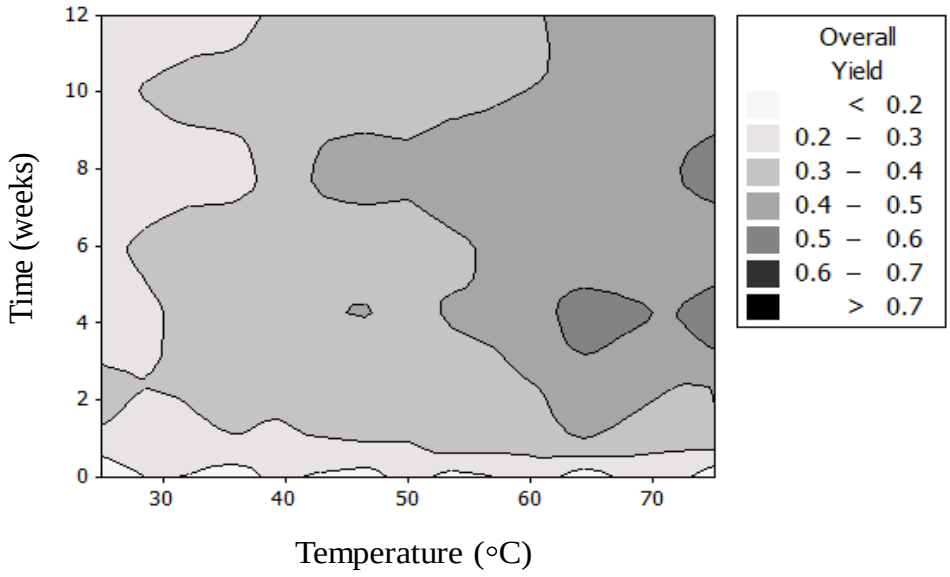


Figure 23. Contour plot for overall yield of HLB as a function of time and temperature. Overall yield is expressed as g glucan+xylan recovered/g glucan+xylan in raw biomass.

Comparing HLB and LLB overall yields. Differences in combined overall yield for HLB and LLB are negligible (Figure 24). A mean difference (LLB yields minus HLB yields) of 0.023 ± 0.14 was observed; thus, the effect of batch on overall yield is not significant. As discussed in Section *Comparing HLB and LLB pretreatment yields*, pretreatment yields of lignin, glucan, and xylan for HLB were different from those of LLB. In particular, lignin degradation was more extensive for LLB; however, LLB was as digestible as HLB. This phenomena is explained because only some delignification (50% according to Zhu et al.[23]) is required to remove the hindrance of lignin to enzyme attack; further delignification is not necessary. Besides, xylan degradation was more extensive and glucan was better preserved in the pretreatment for LLB than in the case of HLB.

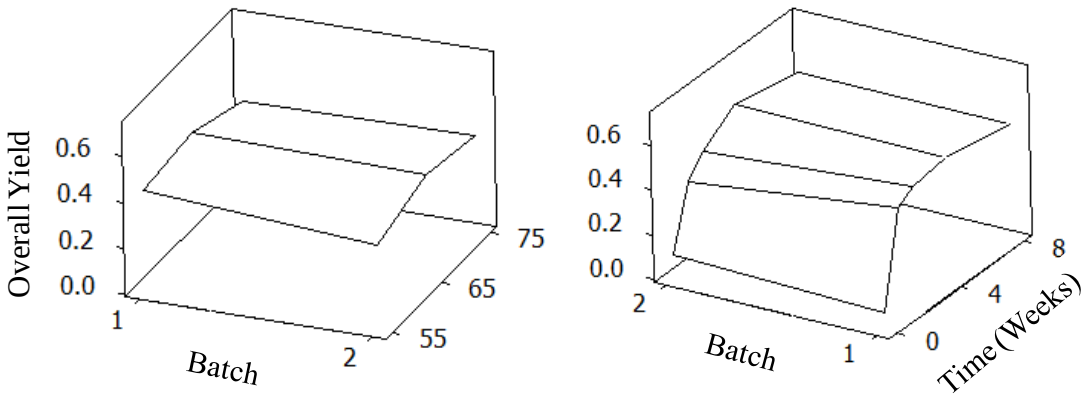


Figure 24. Effects of temperature, batch, and time on overall yield of combined glucan and xylan expressed as g glucan+xylan recovered per g glucan+xylan in raw biomass.

As a consequence of all of these pretreatment and enzymatic yields, overall combined glucan+xylan yields were comparable for both batches.

In another statistical analysis, the effects of time and temperature were separately tested from the effect of batch on combined overall yield. An Anova showed the following *p*-values for a test on the effects of time, temperature, and batch: <0.001, 0.056, and 0.331, respectively. Consequently, although the effects of time and temperature are significant within α = 6%, the effects of batch are unimportant. With these results, the discussion for HLB in Section *Enzymatic and overall hydrolysis yields* can be extended to LLB.

Regarding temperature, 75°C gave higher delignification but because sugar degradation was also greater, the overall result is that 65°C gives better overall yields.

Conclusions

Long-term lime pretreatment produces significant changes in poplar wood composition, mainly reducing lignin and hemicellulose. The extent of change is a function of time, temperature, and aeration. Xylan pretreatment yield is strongly influenced by time, whereas temperature and aeration have a much smaller effect.

Lime consumption is linearly related to lignin and carbohydrate degradation with better fit and greater slopes for the aerated mode. To achieve a given delignification, less lime is required in the aerated mode than in the non-aerated mode.

Biomass pretreated with aeration for more than two weeks at 65°C and submitted to subsequent enzymatic hydrolysis with 15 FPU/g glucan in raw biomass showed an

average improvement of ~5 fold in digestibility. For aerated pretreatment at 65°C and 4 weeks, the improvement was 7.5 fold (0.76 g carbohydrates recovered per g carbohydrates in raw biomass).

Differences in raw poplar lignin content (21.4% and 29.1%) for different batches of the feedstock gave different pretreatment yields, but overall yields were comparable.

SHORT-TERM LIME PRETREATMENT OF POPLAR WOOD*

Synopsis

Short-term lime pretreatment uses lime and high-pressure oxygen to significantly increase the digestibility of poplar wood. When the treated poplar wood was enzymatically hydrolyzed, glucan and xylan were converted to glucose and xylose, respectively. To calculate product yields from raw biomass, these sugars were expressed as equivalent glucan and xylan. To recommend pretreatment conditions, the single criterion was the maximum overall glucan and xylan yields using a cellulase loading of 15 FPU/g glucan in raw biomass. On this basis, the recommended conditions for short-term lime pretreatment of poplar wood follow: (1) 2 h, 140°C, 21.7 bar absolute and (2) 2 h, 160°C and 14.8 bar absolute. In these two cases, the reactivity was nearly identical, thus the selected condition depends on the economic trade-off between pressure and temperature. Considering glucose and xylose and their oligomers produced during 72 h of enzymatic hydrolysis, the overall yields attained under these recommended conditions follow: (1) 95.5 g glucan/100 g of glucan in raw biomass and 73.1 g xylan/100 g xylan in raw biomass and (2) 94.2 g glucan/100 g glucan in raw biomass and 73.2 g xylan/100 g xylan in raw biomass. The yields improved by increasing the enzyme loading. An optimal enzyme cocktail was identified as 67% cellulase, 12% β-glucosidase, and 24%

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xylanase (mass of protein basis) with cellulase activity of 15 FPU/g glucan in raw biomass and total enzyme loading of 51 mg protein/g glucan in raw biomass. Ball milling the lime-treated poplar wood allowed for 100% conversion of glucan in 120 h with a cellulase loading of only 10 FPU/g glucan in raw biomass.

Introduction

For over a hundred years, alkaline pretreatment of wood chips has been employed in paper pulping[129, 130] to remove nearly all lignin. Unfortunately, the severe conditions result in significant carbohydrate losses. [95] Less severe alkaline pretreatments such as alkaline wet oxidation[96, 131, 132] and lime pretreatment have been applied to lignocellulosic biomass resulting in moderate delignification with little or no loss in carbohydrates. Because lignin is known to block enzymes from reaching and hydrolyzing polysaccharides,[23] this outcome is significant for biomass bioconversion processes.

In lime pretreatment, lignocellulosic biomass is mixed with lime (i.e., calcium hydroxide or oxide) and water. As a pretreatment agent, lime is advantageous because it is inexpensive, easily recovered, safe to handle, and compatible with oxidants.[133] This last feature is important because the presence of an oxidative agent such as air or oxygen significantly enhances delignification. The required pretreatment time, temperature, and pressure may vary widely depending on the feedstock lignin content. Selected previous results obtained for temperatures above 100ºC follow:

Low- or medium-lignin biomass (15% to 22%) is rendered digestible without requiring an oxidative agent. For example, the 3-day digestibility of corn stover (21.5% lignin initially), lime treated at 120°C for 4 h without oxygen increased nine times compared to the raw material.[93] Lime pretreatment of switchgrass (21.7% lignin initially) at 100°C for 2 h without oxygen, increased the 3-day total sugar yield 7 times.[77] Bagasse (22% lignin initially) lime treated at 120°C for 1 h without oxygen showed a 3-day corrected sugar reducing yield that was 4.3 times higher that raw bagasse.[91]

High-lignin biomass (23% to 30%) benefits from adding high-pressure oxygen. For example, poplar wood (28% lignin content initially) pretreated at 150°C for 6 h with 14-bar oxygen increased the 3-day reducing sugar yield 9 times compared to raw material and newspaper treated at 140°C for 3 h with 7.1-bar oxygen improved the 3-d reducing sugar yield 2.4 times.[86] The amount of oxygen consumed during these literature pretreatments was not measured.

The aim of the work presented here is to assess short-term lime pretreatment of poplar wood under oxidative conditions. This study will validate the previous results and present them in a format that allows meaningful comparisons with other pretreatment methods evaluated by the Consortium for Applied Fundamentals and Innovation (CAFI). During this study, a novel contribution was made by providing means to hold a constant oxygen pressure in the pretreatment reactors. The results were compared with the older method in which the oxygen pressure was not held constant.

Materials and methods

Substrate. Hybrid poplar wood feedstock (var NM6, genotype P. nigra x P. maximowiczii) was graciously provided by the National Renewable Energy Laboratory (NREL) in two batches. Before shipping, NREL prepared the material by debarking and reducing particle size to pass a ¼-inch round screen. The reduction of particle size was accomplished by chipping, and then milling using an NREL-owned Mitts and Merrill Model 10×12 knife mill (Saginaw, MI). The milled material was then thoroughly mixed by the cone-and-quarter method and was subdivided into 5-gallon pails. Once received in our laboratory, it was re-packaged into Zip-Loc bags (either completely filled or tightly wrapped to reduce moisture evaporation into the headspace), and stored frozen at –20°C. When needed, the biomass was slowly thawed at room temperature and air dried. The particle size was then further reduced to pass 20 to 80 (ASTM) mesh (Fisherbrand U.S. Standard Brass Test Sieves, 12-in dia. × 3-1/4-in depth) using a Fisherbrand Thomas Wiley mill with the purpose of assuring uniformity and reproducibility.

As reported by NREL, the batch of raw poplar wood used in this study was 43.80% glucan, 14.85% xylan, 3.94% mannan, 29.12% lignin, 1.27% galactan, 0.69% arabinan, 3.56% extractives, 1.07% ash, and 3.62% acetyl groups. These values were used as basis for the calculated results presented in this paper.

Experimental setup and operation. Pretreatment was performed in a system of six reactors constructed from 5-in-long, 1.5-in-inside-diameter, 304-stainless-steel pipe

nipples with a 145-mL volume. These reactors were sealed at both ends using Teflon tape and 1.5-in 304-stainless-steel caps. Four temperatures were tested: 110, 140, 160, and 180°C at pressures of 7.9, 14.8, 21.7, and 28.6 bars (absolute) for pretreatment times of 1, 2, 4, 6 and 10 h. Oxygen was used to pressurize the reactors in either one of two modes: varying pressure (VP) in which a single charge of oxygen was added to the reactor at the beginning of the pretreatment process, and constant pressure (CP) in which oxygen was continuously provided during pretreatment at the desired pressure. Constant pressure was attained by using flexible tubing (1/16-in. stainless steel) connected to an oxygen tank (Figure 25).

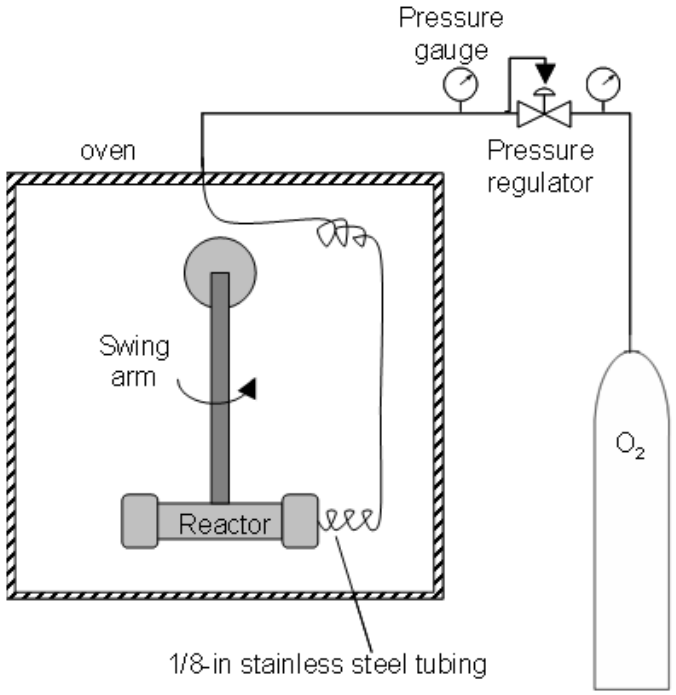

Figure 25. Constant-pressure (CP) lime pretreatment apparatus.

Mixing was provided using a rotating shaft at 10 to 30 rpm (VP case) or a swinging arm (CP case). The pretreatment temperature was maintained by inserting the reactors in a temperature-controlled oven (Fisher Scientific, Isotemp, standard laboratory ovens). To perform the pretreatment, the oven was pre-heated to the desired temperature.

Raw biomass (8 g dry weight) and excess calcium hydroxide (powder, certified, Fisher chemical) (0.4 g/g dry biomass) were placed in each of the six reactors. The mixture was thoroughly mixed with distilled water (15 g/g dry biomass).

After tightly capping and connecting to the manifold, the reactors were placed inside the oven and were exposed to the pretreatment temperature for 10 min of preheat before starting to account for the pretreatment time; this is because while placing the pretreatment apparatus inside the oven and connecting it to the oxygen line, the oven temperature dropped (the oven door was kept open). Pretreatment time was started when the oven (not the reactor) was at the pretreatment temperature. A temperature profile for the CP case and pretreatment temperature of 140ºC is provided in Figure 26. To obtain the profile, a bimetal stem thermometer (from McMaster Carr) was hermetically screwed to the cap of one reactor and the temperature was recorded every 2 to 5 min.

At the end of the pretreatment, the reactors were cooled in a water-ice bath, depressurized by slowly unscrewing the caps, and the pretreated biomass was transferred to a 1-L centrifuge bottle using about 250 mL of deionized (DI) wash water. The slurry was then neutralized by titrating with 5.0-N HCl (Ricca Chemicals) to measure unreacted lime.

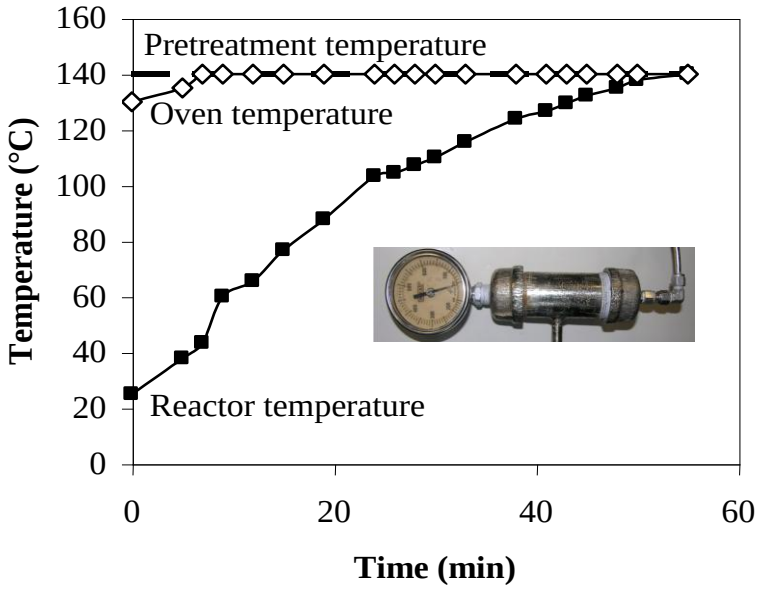

Figure 26. Reactors and oven temperature profile. Target temperature: 140ºC.

The pretreatment liquor was harvested for analysis and the solids were extensively washed with clear DI water and filtered using a vacuum filtration apparatus using a Whatman 934/AH glass fiber filter paper (particle retention = 1.5 µm, Fisher Scientific Co., Pittsburgh, PA). Once filtered, the biomass to be analyzed for composition was air dried at room temperature. The weight of the dry biomass and its moisture content were recorded to account for the pretreatment yield of solids. The biomass was stored at –20°C until used for analysis or enzyme hydrolysis. A total of 105 different conditions of oxidative short-term lime pretreatment were evaluated.

Compositional analysis. Samples of raw and treated poplar wood were prepared for compositional analysis by air drying to a moisture content less than 10% and grinding to a particle size that passed a 20-mesh screen but was retained on an 80-mesh

screen (–20/+80 mesh fraction).[114] The biomass was then extracted with 95% ethanol for 24 h in a Soxhlet apparatus (Pyrex Brand with Allihn Condenser Flask).[115] The extracted samples were acid hydrolyzed in two stages to determine the carbohydrate, lignin, and acetic acid contents. The analysis for carbohydrates and acetic acid were performed by HPLC using Bio-Rad Aminex HPX-87P and Bio-Rad Aminex HPX-87H columns, respectively, with refractive index detection (LabIndex 2000L Refractive Index Detector).[113] The ash content was determined by weighing the sample before and after ashing at 575±25°C (Fisher Scientific, Isotemp, programmable muffle furnace).[116]

Sugar analysis in the pretreatment liquor. When pretreatment was finished, the pretreatment liquor was separated from the biomass through vacuum filtration. It was then collected and the monomeric sugar content and degradation products were quantified by HPLC using a Bio-rad Aminex HPX-87P column with refractive index detection (LabIndex 2000L Refractive Index Detector). Also, a sample of the pretreatment liquor was submitted to acid hydrolysis with 4% sulfuric acid using an autoclave at 125ºC for 1 h. The resulting hydrolyzate was analyzed using HPLC to determine the oligomeric sugar content.[118]

Enzymatic hydrolysis. The substrates used were raw and pretreated-neutralized-washed poplar wood. The cellulase (Spezyme CP®, lot 301-04075-054) and xylanase (Multiefect xylanase®, lot 301-04021-015) used in this study were kindly provided by Genencor International, Inc®. The β-glucosidase (Novozyme 188®, 288 CBU/g of

activity) was obtained from Sigma-Aldrich. Cellulase activity was monitored on a regular basis using the NREL Standard Analytical Procedure.[119] The activities for the xylanase and β-glucosidase were provided by NREL and Novo Nordisk Biochem, respectively.

The required quantity of cellulase was calculated based on its activity, the amount of glucan in the raw biomass, and the desired enzyme loading. The amount of biomass to be weighed was calculated based on the moisture content,[134] the glucan content, and the pretreatment yield of the substrate to provide 0.1 g glucan for the reaction. Water, sodium citrate buffer (0.1 M, pH 4.8), antibiotics (tetracycline, 10 mg/mL in 70% ethanol and cycloheximine, 10 mg/mL in distilled water) and the appropriate mixture of enzymes were added to the substrate to bring the total volume of the mixture to 10 mL .[120] Glucose and xylose concentrations were measured after 72 h of hydrolysis at 50°C in a shaking incubator (Amerex Instruments Inc, Laffayette, CA, 80 rpm). The resulting concentrations were recalculated as glucan and xylan to report yields on the basis of glucan and xylan in raw biomass. To obtain the best cocktail of enzymes, the yields (expressed as mentioned above) were measured after 8, 24, 48, 72 and 180 h of hydrolysis. In both cases, all measurements were performed by HPLC using a Bio-Rad Aminex HPX-87P column with refractive index detection (LabIndex 2000L Refractive Index Detector).

Ball mill. The pretreated poplar wood substrate was ball milled because this process significantly decreases lignocellulose crystallinity thereby increasing its

digestibility.[23] Ball milling was accomplished using a rotary ball mill built with two 1/6-hp 156-rpm AC gearmotors (Dayton Electric Mfg. Co., Niles, IL). The ball mill consists of four 1-in-diameter × 25-in-long steel blower shafts enclosed with 1.5-in O.D. Buna-N rubber tubing (McMaster-Carr, Atlanta, GA). A 300-mL porcelain jar was charged with 0.375-in zirconia grinding medium (ZGM) (U.S. Stoneware, East Palestine, OH) to about 50% of the jar volume (about 258 g of ZGM). Biomass was placed in the jar to fill the void volume between the ZGM. The ratio of ZGM to biomass was 43 g ZGM/g dry biomass. Then, the jars were placed between the rollers and rotated at 68 rpm for 3 days. After pretreatment and ball milling, the poplar wood was enzymatically hydrolyzed with a cellulase loading of 5, 10, 15, 60 and 120 FPU/g glucan in raw biomass, and excess β-glucosidase (60 CBU/g glucan in raw biomass). The hydrolysis conditions were held for 48, 72, and 120 h. The hydrolyzed samples were analyzed for sugars by HPLC using a Biorad HPX-87P column with refractive index detector.

Results and discussion

Lime consumption. Table 7 presents the lime consumption. For the recommended pretreatment conditions, the lime consumption was 0.234 and 0.198 g $Ca(OH)_2$ consumed/g dry biomass for the CP and VP modes, respectively. In general, for higher temperatures, pressures, and pretreatment times, more lime was consumed. Additionally, a linear relationship between lignin removal and lime consumption was observed, with somewhat more lignin removed for a fixed time in the VP mode (Figure 27).

Table 7. Selected results for lime consumption as a function of the pretreatment conditions (CP mode)

Pretreatment conditions			Lime consumed (g/g dry biomass)
Temperature (ºC)	Time (h)	Pressure (bar absolute)	
140	**2**	**21.7**	**0.234[(a)]**
160	**2**	**14.8**	**0.198[(a)]**
110	2	7.9	0.180
		14.8	0.181
		21.7	0.190
110	4	14.8	0.190
140			0.297
160			0.365
180			0.380
180	2	14.8	0.313
	4		0.378
	10		0.390

(a) Lime consumed at the recommended pretreatment conditions.

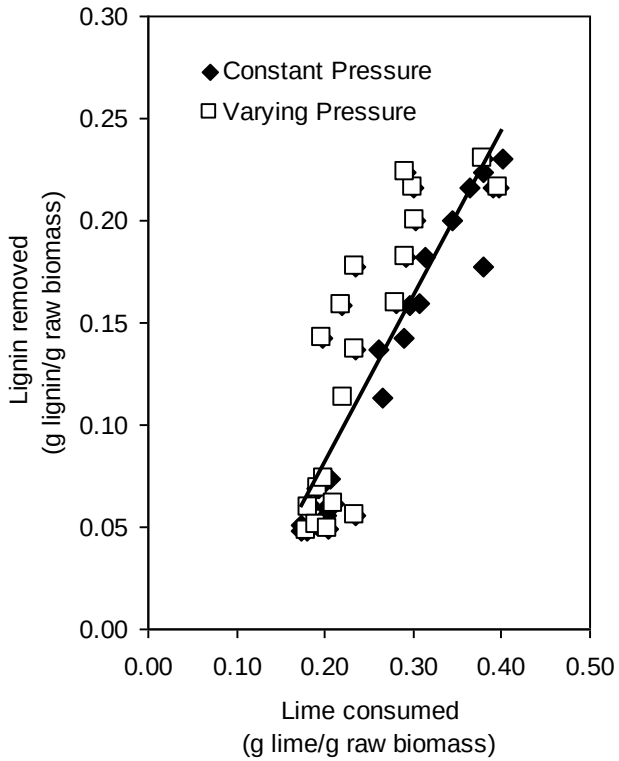

Figure 27. Relationship between lime consumption and lignin removed.

Lime consumption has been reported before for lime pretreatment of corn stover at mild conditions (atmospheric pressure, with bubbling air and from room temperature to 55°C).[75] In this case, the reported specific lime consumption for the recommended pretreat ment condition (4 weeks, with air and 55°C) was only 0.073 g lime consumed/g dry biomass. This result further supports the observation that harsher pretreatment conditions increase lime consumption. The type of biomass under pretreatment also influences lime consumption.

Pretreatment yields. The following discussion emphasizes only typical cases. Results for solids are presented first, then pretreatment yields of liquid.

Solids. The pretreatment yields of interest include: *glucan pretreatment yields* (i.e., glucan remaining in the solids after pretreatment), *xylan pretreatment yields* (i.e., xylan remaining in the solids after pretreatment), and *lignin pretreatment yields* (i.e., lignin remaining in the solids after pretreatment). In general, less lignin and carbohydrates were recovered when the solids underwent aggressive pretreatments (higher temperatures, higher pressures, and longer time). Figure 28 shows selected results. Carbohydrate degradation was slightly higher in the CP mode than in the VP mode, whereas the opposite occurred for lignin. Glucan pretreatment yields typically were above 80 g glucan recovered/100 g glucan in raw biomass (Figures 28a and 28b). However, in some cases, these yields were better.

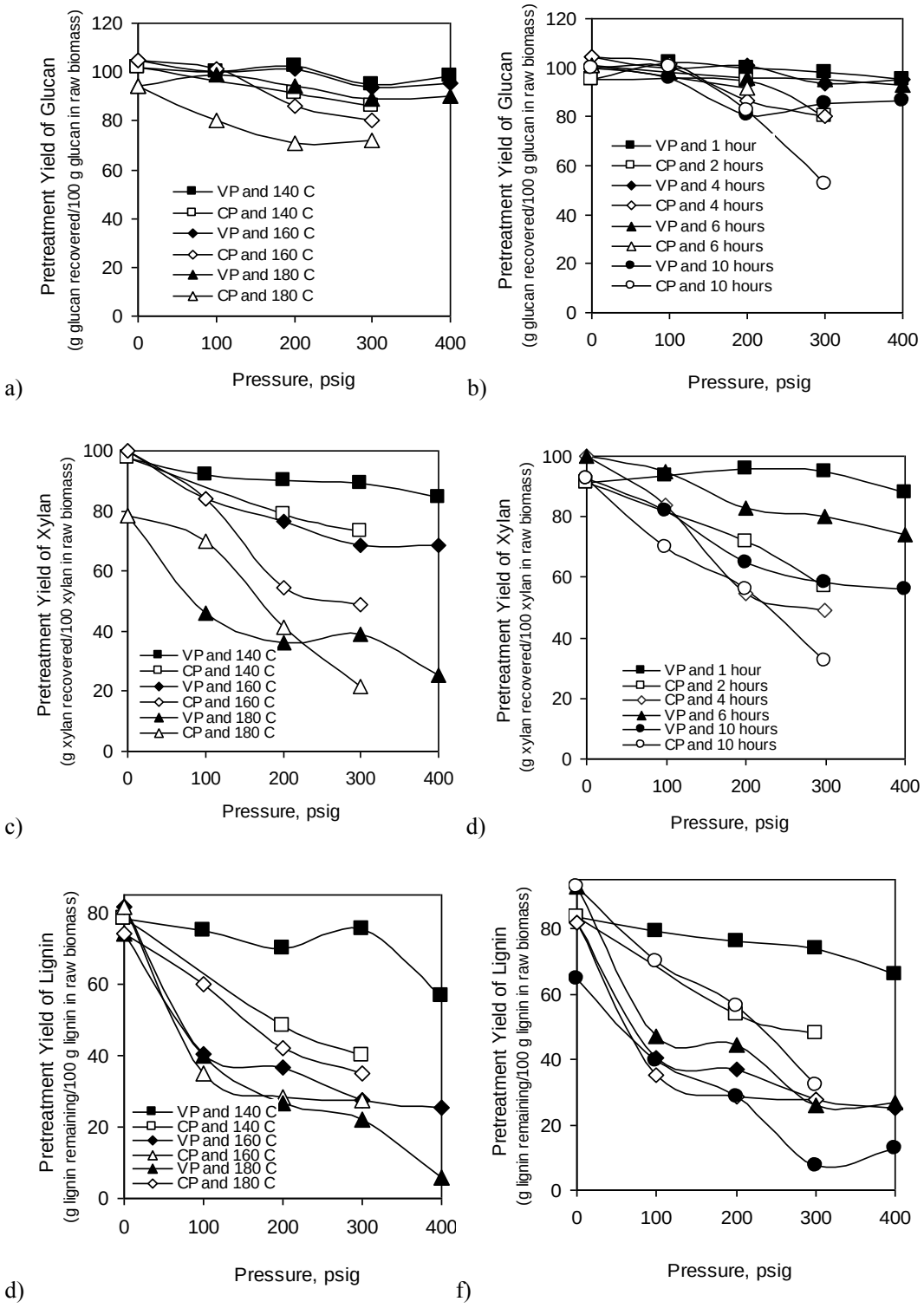

Figure 28. Pretreatment yields (a) glucan 4 h, (b) glucan 160°C, (c) xylan 4 h, (d) xylan 160°C, (e) lignin 4 h, (f) lignin 160°C. (Note: The symbols for Figure 28f are the same as for Figure 28d.)

At 110 and 140°C the glucan yields were above 90 g glucan recovered/100 g glucan in raw biomass. In other cases, the glucan yields were worse. At 160 and 180°C, 10 h, and 21.7 bar (absolute), they were only about 50 g glucan recovered/100 g glucan in raw biomass for both CP and VP modes (not all data shown).

In the case of xylan, the degradation was much faster and more severe (Figures 28c and 28d). At 110 and 140°C, the yields were above 70 g xylan recovered/100 g xylan in the raw biomass. At 160°C, it decreased to a minimum of 50 g xylan recovered/100 g xylan in the raw biomass in the case of CP mode and 10 h. At 180°C for 4 h or more and above 14.8 bar (absolute), the xylan yields were <20 g xylan recovered/100 g xylan in raw biomass. At 180°C for 4 h and 21.7 bar (absolute), xylan yields as low as 9 g xylan recovered/100 g xylan in raw biomass were observed.

For lignin, the degradation was even more drastic (Figures 28e and 28f). At 110 and 130°C, the yields were 40 g of lignin remaining/100 g lignin in the raw biomass. At 160°C, 10 h, and 21.7 bar (absolute), yields were as low as 6 g of lignin remaining/100 g lignin in the raw biomass. At 180°C, only 6 h (VP) are required to achieve this same yield. Interestingly, for temperatures of 160°C or above and times of 4 h or above, VP treatments gave lower lignin yields than the CP treatments (Figures 28e and 28f).

Liquid. The pretreatment liquor was analyzed for sugars after pretreatment. Regardless of the pretreatment conditions, the structural sugars were not found as monomers; however, oligomers (particularly of xylan) were found (see Table 8).

Table 8. Oligomers of glucose and xylose recovered in the pretreatment liquor for diverse conditions of pretreatment

Pretreatment conditions			Oligomers recovered	
Temperature (°C)	Time (h)	Pressure (bar absolute)	Glucose %[(a)]	Xylose %[(a)]
130	4	14.8	0.46	2.17
130	10	14.8	0.51	0.72
130	2	21.7	0.40	2.00
130	4	21.7	0.56	1.05
150	2	14.8	0.55	2.87
150	4	14.8	0.63	3.77
150	10	14.8	0.47	1.07
150	2	21.7	0.49	3.77
150	10	21.7	0.57	0.64
150	4	2.7	0.39	1.76

(a) Percentage expressed as g glucose (xylose) recovered/100 g raw biomass

Accounting for solids in and out of the pretreatment operation, a negative balance for glucan, xylan, and lignin was obtained; thus, some of these components reacted during pretreatment and formed soluble degradation products.

The amount of degradation products was measured by gravimetric analysis only (see mass balance section). No other measurements or efforts to identify the specific nature of degradation products were made because other studies[75, 86, 127] have shown that they do not inhibit enzymatic hydrolysis or fermentation.

Enzymatic hydrolysis yields. The substrates used in this study were raw poplar wood and pretreated-neutralized-washed solids of poplar wood. After enzymatic hydrolysis, glucan and xylan were converted to glucose and xylose, but these were expressed as equivalent glucan and xylan to calculate the yields from raw biomass.

Detailed calculations of all yields have been published elsewhere.[75] Considering the enzymatic hydrolysis operation alone, *glucan (and xylan) enzymatic yields* were obtained (i.e., yields based only on the enzymatic hydrolysis operation); however, the recommended pretreatment conditions were chosen based on the *glucan and xylan overall yield* (i.e., yields after the combined operations of pretreatment and enzymatic hydrolysis).

Substrate was prepared by applying the recommended pretreatment condition to raw poplar, neutralizing, and extensively washing the pretreated solids. Then, these solids were enzymatically hydrolyzed using different enzyme cocktails to determine the loading and enzyme mixture (cellulase, β-glucosidase, and xylanase) that provides the best *glucan+xylan overall yield* (i.e., total glucan plus xylan obtained after the combined operations of pretreatment and enzymatic hydrolysis on the solids). Results obtained from these experiments are summarized below.

Recommended pretreatment conditions. The single criterion used to determine the optimum pretreatment condition was the 72-h glucan and xylan overall yield (defined previously) with a cellulase loading of 15 FPU/g glucan in raw biomass and excess β-glucosidase (60 CBU/g glucan in raw biomass).

Typical cases presented in Figure 29 show that yields strongly depend on pretreatment temperature, time, and pressure. Additionally, similar pretreatment conditions gave different glucan and xylan overall yields depending on the mode (CP or VP).

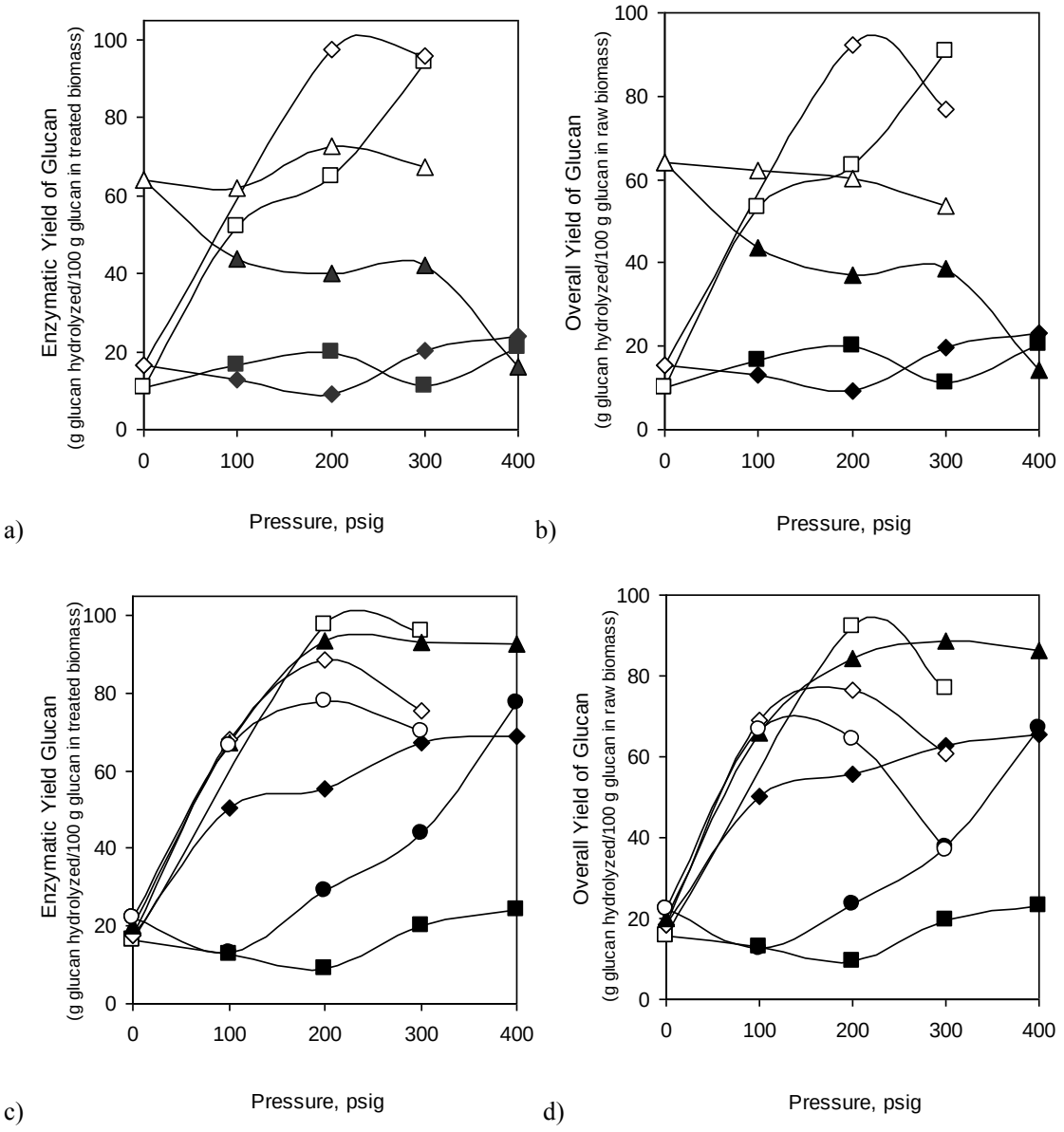

Figure 29. Effect of time, temperature and pressure on the enzymatic yield of glucan. Enzyme hydrolysis conditions: 72 h, 15 FPU/g glucan in raw biomass and 50°C. (a) and (b) correspond to 1 h for VP and 2 h for CP. Figures (c) and (d) correspond to 160°C. The symbols for plots (a) and (b) follow: VP and 140°C (■), CP and 140°C (□), VP and 160°C (◆), CP and 160°C (◊), VP and 180°C (▲), CP and 180°C (Δ). The symbols for plots (c) and (d) follow: CP and 2 h (□), VP and 1 h (■), CP and 4 h (◆), VP and 4 h (◊), VP and 6 h (▲), CP and 10 h (●) and VP and 10 h (○).

At any time or pressure, enzymatic glucan yields obtained for pretreatments at 110°C were lower than 40 g glucan hydrolyzed/100 g glucan in the treated biomass (data not shown). Also, 1-h pretreatment was not enough at any temperature or pressure. However, at 140, 160 and 180°C, and for 2 h or more, high enzymatic and overall glucan yields were observed.

The best glucan yields are summarized in Tables 9 and 10. Glucan yields were always lower in VP than in CP and required higher temperatures and longer pretreatments; thus, the VP mode was not recommended.

Considering all of these results, the recommended pretreatment conditions for poplar wood follow: (1) CP mode, 2 h, 140°C, and 21.7 bar (absolute) and (2) CP mode, 2 h, 160°C and 14.8 bar (absolute). The yields were similar, so the choice depends on economical considerations. Table 9 summarizes the pretreatment, enzymatic, and overall glucan and xylan yields obtained for the recommended cases.

It is interesting to note that in the CP case, xylan yields were lower than glucan yields. In contrast, in the VP case, some instances were found where xylan yields were higher than the corresponding glucan yields (Figure 30).

Nevertheless, the most general cases show xylan yields lower than glucan yields. The following section describes studies that explore if the addition of xylanase to the enzyme cocktail improves xylan and/or glucan yields.

Table 9. Highest enzymatic and overall yields of glucan[(a)] and xylan[(a)] observed after short-term lime pretreatment[(b)]

Pretreatment Conditions	Mode		EGY[(c)]	OGY[(d)]	EXY[(e)]	OXY[(f)]
	CP	VP				
2 h – 140°C – 21.7 bar absolute	×		96	91	89	65
2 h – 160°C – 14.8 bar absolute	×		96	92	90	66
2 h – 160°C – 21.7 bar absolute	×		96	76	88	51
4 h – 180°C – 14.8 bar absolute	×		94	65	90	32
10 h – 140°C – 14.8 bar absolute		×	92	85	90	76
6 h – 140°C – 7.9 bar absolute		×	92	70	78	71
6 h – 160°C – 14.8 bar absolute		×	93	84	94	78
6 h – 160°C – 21.7 bar absolute		×	93	88	94	75
6 h – 160°C – 28.6 bar absolute		×	92	86	96	70

(a) Mass of glucose and xylose expressed as mass of equivalent glucan and xylan to calculate the yield based on glucan and xylan in the feedstock.
(b) Enzyme hydrolysis obtained after 72-h hydrolysis at cellulose loading of 15 FPU/g glucan in raw biomass.
(c) Enzymatic glucan yield (EGY) (g glucan hydrolyzed/100 g glucan in treated biomass)
(d) Overall glucan yield (OGY) (g glucan hydrolyzed/100 g glucan in raw biomass)
(e) Enzymatic xylan yield (EXY)(g xylan hydrolyzed/100 g xylan in treated biomass)
(f) Overall xylan yield (OXY) (g xylan hydrolyzed/100 g xylan in raw biomass)

Ezyme loading study. Poplar wood pretreated at one of the recommended conditions (140°C, 2 h, 21.7 bar absolute) was used as substrate for this study. After pretreatment, the carbohydrate recovery of this sample was 90.4 g (glucan + xylan)/100 g glucan+xylan in raw biomass. Different enzyme cocktails were prepared using cellulase (5, 10, 15, 60 and 120 FPU/g glucan in raw biomass), xylanase (0, 11 and 23 mg/g glucan in raw biomass) and β-glucosidase (6 and 24 mg/g glucan in raw biomass).

Table 10. Pretreatment, enzymatic, and overall yields obtained at the recommended conditions of pretreatment[a]

Yields / Pretreatment conditions	140°C, 2 h 21.7 bar	160°C, 2 h 14.8 bar
Pretreatment Yield of Glucan[b] (g glucan recovered/100 g glucan in raw biomass)	95.9	94.6
Enzymatic Glucan[c] (g glucan hydrolyzed/100 g glucan in treated biomass)	99.6	99.6
Overall Glucan[c] (g glucan hydrolyzed/100 g glucan in raw biomass)	95.5	94.2
Pretreatment Yield of Xylan [b] (g xylan recovered/100 g xylan in raw biomass)	73.4	70.7
Enzymatic Xylan[c] (g xylan hydrolyzed/100 g xylan in treated biomass)	99.6	103.5
Overall Xylan[c] (g xylan hydrolyzed/100 g xylan in raw biomass)	73.1	73.2

(a) Mass of glucose and xylose were expressed as mass of glucan and xylan to make the yield calculation on the basis of glucan and xylan in the feedstock.
(b) Glucose and xylose in the pretreatment liquor were not accounted for in these calculations.
(c) Some glucan and xylan were found available after enzymatic hydrolysis in oligomeric form in the liquor. They were measured and included in these calculations.

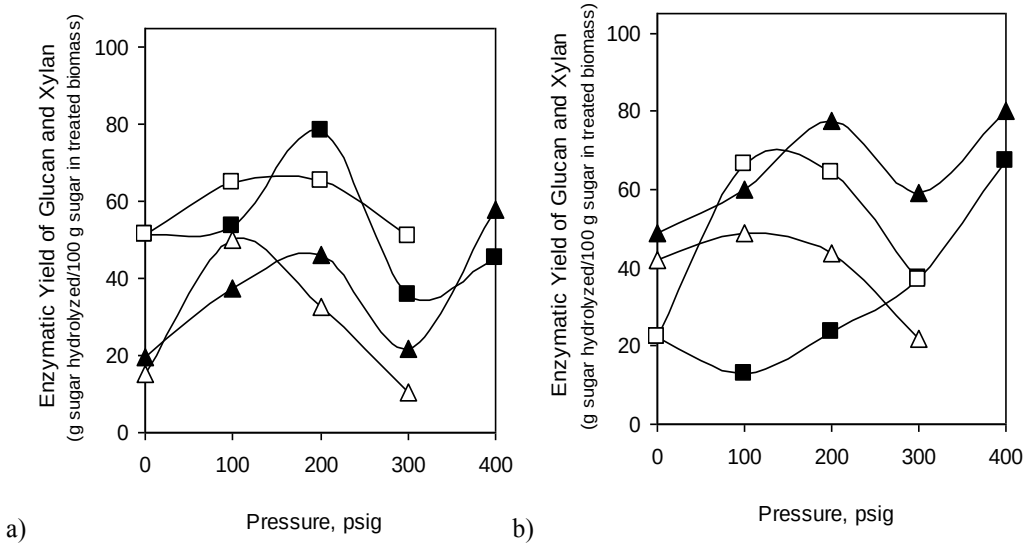

Figure 30. Comparison between the enzymatic (a) and overall (b) yields of glucan and xylan in the CP and in the VP modes at pretreatment conditions of 160°C and 4 h. Symbols: VP and glucan yield (■), CP and glucan yield (□), VP and xylan yield (◆), CP and xylan yield (◊).

Using the procedure explained in Section 2.5 of this paper, the samples were analyzed for sugars after 0, 8, 24, 48, 72 and 180 h of enzymatic hydrolysis.

The criteria used to select the best enzymes cocktail follow: (1) Give an overall yield of glucan + xylan close to the maximum potential overall yield of 90.4 g glucan + xylan hydrolyzed/100 g glucan + xylan in raw biomass. (2) Use the minimum possible amount of enzyme. At <48 h of hydrolysis, all the yields failed to satisfy either of the selection criteria. Hydrolysis for 180 h was not significantly improved compared to hydrolysis for 72 h; therefore, 72 h was selected. At this hydrolysis time, the choice of the best enzyme cocktail was performed on the basis of *enzyme efficiency*, i.e., the ratio between the overall yield of glucan + xylan and the total amount of enzyme loaded.

Several high efficiencies were observed (Table 11). Some high efficiencies occurred at low enzyme loadings but gave only a moderate yield (about 30 to 60 g glucan + xylan hydrolyzed/100 glucan + xylan in raw biomass).

These were ignored because they did not meet Criterion 1. The only enzyme loadings that were considered viable had total yields >80 g glucan + xylan hydrolyzed/100 g glucan + xylan in raw biomass. One enzyme cocktail with a cellulase loading of 15 FPU/g glucan in raw biomass gave a total yield of 82 g glucan + xylan hydrolyzed/100 g glucan + xylan in raw biomass. The total protein loading was 51 mg protein/g glucan in raw biomass. On a protein basis, the composition of this cocktail was 67% cellulase, 12% β-glucosidase, and 24% xylanase.

Table 11. Enzyme efficiency for all the different enzyme cocktails tested at 72-h hydrolysis.

Enzymes loaded (mg/g glucan in raw biomass)				Overall Yield[f]			
Cellulase	β-glucosidase	Xylanase	Total	Glucan[a]	Xylan[b]	Total[c]	Efficiency[e]
11 (5)[d]	6	0	17	33.0	4.4	37.4	2.22
		12	29	20.3	4.1	24.4	0.85
		23	40	25.6	4.3	29.9	0.75
	24	0	35	35.2	7.0	42.1	1.20
		23	58	32.6	5.6	38.2	0.66
22 (10)[d]	6	0	28	49.0	5.8	54.7	1.95
		12	40	34.1	5.6	39.7	0.99
		23	51	57.6	9.7	67.3	1.32
	24	0	46	39.5	7.8	47.3	1.02
		23	69	53.1	7.1	60.3	0.87
34 (15)[d]	**6**	0	39	56.7	5.7	62.4	1.59
		12	**51**	**72.9**	**9.3**	**82.2**	**1.60**
		23	62	65.3	12.8	78.2	1.26
	24	0	57	67.4	13.4	80.8	1.41
		23	80	33.1	10.6	43.7	0.54
134 (60)[d]	6	0	140	71.8	9.0	80.8	0.58
		12	152	71.8	11.6	83.4	0.55
		23	163	71.9	12.1	84.0	0.52
	24	0	158	68.2	11.2	79.4	0.50
		23	181	70.4	18.6	90.0	0.50
268 (120)[d]	6	0	274	71.8	15.8	87.6	0.32
		23	297	71.8	12.5	84.3	0.28
		12	286	72.6	14.0	86.5	0.30
	24	23	315	71.8	18.6	90.4	0.29

(a) g glucan hydrolyzed/100 g glucan + xylan in raw biomass
(b) g xylan hydrolyzed/100 g glucan + xylan in raw biomass
(c) g glucan + xylan hydrolyzed/100 g glucan + xylan in raw biomass
(d) The number in the parentheses is the cellulase loading in FPU/g glucan in raw biomass.
(e) Efficiency = Column 7/Column 4
(f) Includes monomers only.

Ball milling pretreated poplar wood. Poplar wood that had undergone lime pretreatment at one the recommended conditions (140ºC, 2 h and 21.7 bar absolute) was air dried and then ball milled for 3 days using the procedure explained in Materials and Methods. The resulting ball-milled material was then used as substrate for enzymatic hydrolysis with cellulase loadings of 5, 10, 15 and 60 FPU/g glucan in raw biomass (11,

22, 34 and 134 mg protein/g glucan in raw biomass respectively) and excess β-glucosidase (60 CBU/g glucan in raw biomass, which is 24 mg protein/g glucan in raw biomass). Xylanase was not added. The enzymatic conversion was determined by measuring the sugar content in the hydrolyzate obtained after enzymatic hydrolysis. After enzymatic hydrolysis, the hydrolyzate was not tested for presence of undigested glucan or xylan oligomers. All yields depended on enzyme loading and hydrolysis time (Figure 31). With pretreated ball milled poplar, complete hydrolysis was possible in 120 h with an enzyme loading of only 10 FPU/g glucan in raw biomass. With pretreated-only material, complete hydrolysis was not possible at any time or enzyme loading; thus, the digestibility of poplar wood was significantly increased by ball milling. Because the most important effect of ball milling is to reduce crystallinity,[23] these results show the important effect of crystallinity on biomass digestibility. In all cases β-glucosidase was added in excess (60 CBU/g glucan in raw biomass) but no xylanase was added.

Mass balances. Mass balances were obtained for the two recommended pretreatment conditions, i.e., 140°C, 2 h, 21.7 bar (absolute) and 160°C, 2h, 14.8 bar (absolute) (Tables 12 and 13 respectively). The mass balances closed within 98% and 97%, respectively. Lignin and xylan generated a significant amount of degradation products (20.45 and 22.71 g/100 g raw biomass), which were found in the pretreatment liquor. The enzymatic hydrolysis liquor contains primarily monomers of glucan and xylan, but also some oligomers.

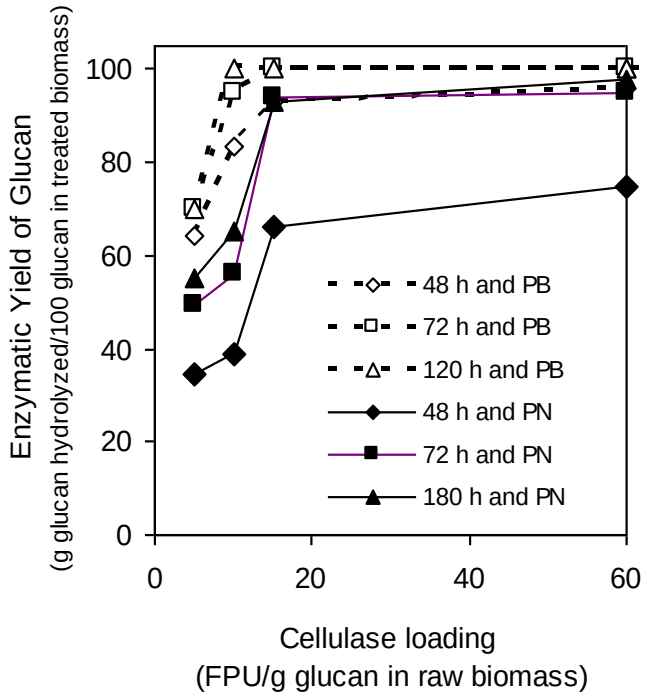

Figure 31. Digestibility of pretreated and ball milled (PB) and pretreated and not ball milled (PN) poplar wood at different cellulase loadings and times of hydrolysis.

Conclusions

The recommended pretreatment conditions are (1) 140°C, 2 h, and 21.7 bar (absolute) or (2) 160°C, 2 h, 21.7 bar (absolute), both in the CP mode. The glucan and xylan pretreatment yields obtained in these cases are 95.9 g glucan recovered/100 g glucan in raw biomass and 73.4 g xylan recovered/100 g xylan in raw biomass for the former and 94.6 g glucan recovered/100 g glucan in raw biomass and 70.7 g xylan recovered/100 g xylan in raw biomass for the later.

Using an enzyme loading of 15 FPU/g glucan in raw biomass and 72 h of hydrolysis, the enzymatic pretreatment yields are 99.6 g glucan hydrolyzed/100 g glucan in treated biomass and 99.6 g xylan hydrolyzed/100 g xylan in treated biomass for the

Table 12. Mass balances from raw poplar wood to pretreated[(a)] and hydrolyzed[(b)] at 140 ºC

Component	Raw	Mass after pretreatment (kg)			Mass after enzyme hydrolysis (kg)		
		Solids	**Liquid**	**Total**	**Solids**	**Liquid**	**Total**
Glucan	43.80	42.01	0.15	42.16	0.18	39.60	39.78
Glucan left as oligomers						2.23	2.23
Xylan	14.85	10.90	0.66	11.56	0.00	9.70	9.70
Xylan left as oligomers						1.16	1.16
Mannan	3.94	1.68	0.09	1.77	0.29	1.39	1.68
Lignin	29.12	16.25	4.96	21.21	6.54	9.71	16.25
Others	10.21	2.85[(d)]	0.00	2.85	2.85	0.00	2.85
Degradation products			20.45[(e)]	20.45			
Total	**101.92**	**73.69[(c)]**	**26.31**	**100**	**9.86**	**63.79**	**73.65**

Mass balance closure (73.69 + 26.31)/101.92 × (63.79 + 9.86)/73.69 = 98.06%

[(a)] Conditions of pretreatment: 140°C, 2 h, 300 psig, CP.

[(b)] Conditions of hydrolysis: 15 FPU/g glucan in raw biomass, 50°C, 72 h.

[(c)] Measured gravimetrically

[(d)] Includes arabinan, galactan, extractives, ash, and acetyl. Calculated as Total PS minus lignin and carbohydrates in PS. i.e., 73.69 – 16.25 – 1.68 – 10.90 – 42.01 = 2.85

[(e)] Calculated as 100 minus all the other weights in pretreatment liquid and pretreatment solids, i.e., 100 – (4.96 + 0.09 + 0.67 + 0.14) – 73.69 = 20.45

Table 13. Mass balances from raw poplar wood to pretreated(a) and hydrolyzed(b) at 160 ºC

Component	Raw	Mass after pretreatment (kg)			Mass after enzyme hydrolysis (kg)		
		Solids	**Liquid**	**Total**	**Solids**	**Liquid**	**Total**
Glucan	43.80	41.45	0.14	41.59	0.18	40.49	40.67
Glucan left as oligomers						0.78	0.78
Xylan	14.85	10.50	0.67	11.67	0.01	9.71	9.72
Xylan left as oligomers						1.16	1.16
Mannan	3.94	1.80	0.08	1.88	0.31	1.42	1.73
Lignin	29.12	15.95	3.97	19.92	6.30	9.65	15.95
Others	10.21	2.73 [(d)]	0.00	2.73	1.80	0.00	1.80
Degradation products			22.71 [(e)]	22.71			
Total	**101.92**	**72.43[(c)]**	**27.57**	**100.5**	**8.60**	**63.21**	**71.81**

Mass balance closure (72.43 + 27.57)/101.92 × (63.21 + 8.60)/72.43 = 97.28%

[(b)] Conditions of hydrolysis: 15 FPU/g glucan in raw biomass, 50°C, 72 h.

[(c)] Measured gravimetrically

[(d)] Includes arabinan, galactan, extractives, ash, and acetyl. Calculated as Total pretreatment solids minus lignin and carbohydrates in PS. i.e., 72.43 – 15.95 – 1.80 – 10.50 – 41.45 = 2.73

[e] Calculated as 100 minus all the other weights in pretreatment liquor and pretreatment solids, i.e., 100 – (3.97 + 0.08 + 0.67 + 0.14) – 72.43 = 22.71

former and 99.6 g glucan hydrolyzed/100 g glucan in treated biomass and 103.5 g xylan hydrolyzed/100 g xylan in treated biomass for the later.

Finally, the overall glucan and xylan yields (i.e., glucose and xylose recovered after both pretreatment and enzymatic hydrolysis, and expressed as equivalent glucan and xylan) for the former condition are 95.5 g glucan/100 g of glucan in raw biomass and 73.1 g xylan/100 g xylan in raw biomass and for the later condition are 94.2 g glucan/100 g glucan in raw biomass and 73.2 g xylan/100 g xylan in raw biomass. Respectively, these yields are equivalent to 90.1 and 89.2 g glucan+xylan/100 g glucan+xylan in raw biomass. During the recommended pretreatment conditions, the lime consumption was 0.234 and 0.198 g $Ca(OH)_2$ consumed/g dry biomass, respectively. Mainly lignin and xylose were dissolved in the pretreatment liquor and were degraded to some extent during pretreatment, in a quantity proportional to the pretreatment time, temperature, pressure, and mode (CP or VP). An enzyme cocktail containing cellulase (67%), xylanase (24%), and β-glucosidase (12%) was most efficient using a protein loading of 51 mg protein/g glucan in raw biomass. Using pretreatment plus ball milling, it was possible to achieve full conversion of the sugars remaining in the solid after the recommended pretreatment with low enzyme loadings (10 FPU/g glucan in raw biomass) and no addition of xylanase.

SELECTIVITY AND DELIGNIFICATION KINETICS FOR OXIDATIVE SHORT-TERM LIME PRETREATMENT OF POPLAR WOOD. PART I: CONSTANT-PRESSURE

Synopsis

Kinetic models applied to oxygen bleaching of paper pulp focus on the degradation of polymers, either lignin or carbohydrates. Traditionally, they separately model different moieties that degrade at three different rates: rapid, medium, and slow. These models were successfully applied to lignin and carbohydrate degradation of poplar wood submitted to oxidative pretreatment with lime at the following conditions: temperature 110 to 180°C, total pressure 7.9 to 21.7 bar, and excess lime loading of 0.5 g $Ca(OH)_2$/g dry biomass. These conditions were held constant for 1 to 6 hours. The models properly fit experimental data and were used to determine pretreatment selectivity in two fashions: differential and integral. By assessing selectivity, the detrimental effect of pretreatment on carbohydrates at high temperatures and at low lignin content was determined. The models can be used to identify pretreatment conditions that selectively remove lignin while preserving carbohydrates. Lignin removal ≥50% with glucan preservation ≥90% was observed for differential glucan selectivities between ~10 and ~30 g lignin degraded/g glucan degraded.

Pretreatment conditions complying with these reference values were preferably observed at 140°C, total pressure ≥14.7 bars, and for pretreatment times between 2 and 6 hours depending on the total pressure (the higher the pressure, the less time). They were

also observed at 160°C, total pressure of 14.7 and 21.7 bars, and pretreatment time of 2 hours. Generally, at 110°C lignin removal is insufficient and at 180°C carbohydrates do not preserve well.

Introduction

Modern paper mills often use oxygen delignification or oxygen bleaching because oxygen is more cost-effective than other traditionally used chemicals and because it is environmentally safe. This process uses temperatures of 80 to 100°C, oxygen pressures of 5 to 6 bars, and alkaline conditions (pH > 10) obtained by adding NaOH.[100, 104, 106] As a result, about 50% of the residual lignin is removed. In oxygen delignification degradation of carbohydrates and lignin is caused by active oxygen species that are secondary reaction products of lignin and molecular oxygen.

To biologically produce fuels and chemicals, lignocellulose may undergo oxidative lime pretreatment. Through this pretreatment, partial delignification of biomass is obtained allowing for some biomass swelling, increased internal surface area, and larger median pore volume, all of which enhance enzyme accessibility to carbohydrate polymers.[23, 24, 111] After pretreatment, the biomass can be saccharified and fermented to fuels and chemicals, or used directly as animal feed, if properly enriched with nutrients.

Large amounts of data have been generated on delignification kinetics applied to oxygen bleaching.[135-139] Building on this background, this study develops delignification kinetic models for oxidative lime pretreatment of poplar wood. These models can be

used for the following purposes: (1) to gain insight into the lime pretreatment process, (2) to design commercial-scale equipment, and (3) for process control and optimization.

To react with lignin, oxygen requires high temperatures; however, according to previous studies, to preserve carbohydrates, the temperature must be less than 120°C.[137] The aim of this study is to model lignin and carbohydrates degradation in oxidative alkaline media at temperatures from 110 to 180°C. The resulting equations are used to calculate selectivity. High pressure is used to improve oxygen solubility in the liquid phase. In this work, oxygen concentration is maintained constant by open oxygen lines directly connected to reaction vessels.

This article is part of a four-paper series that describes the results of kinetic modeling oxidative lime pretreatment of poplar wood with the following topics: (I) constant-pressure pretreatment (this study); (II) varying-pressure pretreatment,[140] where oxygen is feed to the desired pressure only at the beginning of pretreatment; (III) low-temperature and long-term pretreatment,[126] in open vessels with and without bubbling air, at temperatures up to 75°C; and (IV) comparison of all results obtained to recommend pretreatment conditions that optimize selectivity.[112]

Delignification mechanisms

The reaction of oxygen requires the release of electrons, which is promoted by ionizing functional groups in a strongly alkaline media. At high pH, oxygen is reduced by one electron transfer to a number of different oxidizing species (radicals), each with different reactivity.[101, 137 141] These free radicals attack biomass components by

introducing hydrophilic groups into the lignin structure that break inter-unit linkages, thereby increasing lignin solubility. Nucleophilic attack from a hydroxyl peroxide anion may also occur, resulting in ring opening. Condensation products can result through coupling reactions between phenoxy radicals that produce carbon-carbon bonds between lignin units. These reactions make the lignin unreactive to oxygen attack.[142]

Concurrently, "peeling" delignification occurs at the reducing ends where hemicellulose is covalently bonded to lignin. Carbohydrate-derived radicals react rapidly with oxygen to give carbonyl structures (glucosones) and other oxidation products. The formation of carbonyl structures in cellulose may lead to alkali-induced cleavage of glucosidic bonds (''cellulose peeling''); hence, hydroxyl radicals may cause both direct and indirect cleavage of glucosidic linkages in cellulose.[100]

In alkaline oxidative delignification, gas-to-liquid and liquid-to-solid mass transfers must be considered. Extensive publications provide details and explain experimentally observed phenomena, such as decreased efficiency of oxygen delignification at increased lignin removal.[101, 106]

Methods

Kinetic data were collected using 145-mL batch reactors loaded with 8 g (dry weight) of poplar wood, 4 g of lime [$Ca(OH)_2$], and 120 g of water. These reactors, made of 304 stainless steel nipples, were sealed at both ends using Teflon tape and 1.5-inch 304 stainless steel caps, as reported elsewhere.[111] Four temperatures were tested: 110, 140, 160, and 180°C. To start pretreatments, the reactors were filled with the

biomass, lime, and water. After mixing well and closing tightly, the reactors were placed inside a preheated oven and then connected through a manifold to an oxygen line that was then set to the desired pressure (7.9, 14.8, or 21.7 bars absolute). The reactor pressure was held constant at all times during pretreatment. Mixing was provided through a swing arm moving at 30 rpm to which the reactors were attached.

Lignin and carbohydrate measurements of solids were performed according to National Renewable Energy Laboratory Analytical Procedures.[113, 115, 116, 118] Additional details on the experimental setup, analytical methods, and an example of reactor temperature profile have been published elsewhere.[111]

The measurements were reported in terms of lignin, glucan, and xylan yield, which are defined as follows:

$$Y_i \equiv \frac{C_i \cdot Y_T}{C_{i_0}} \tag{3}$$

where

i = lignin L, glucan G, or xylan X

Y_i = pretreatment yield of Component i at time t (kg residual Component i/kg Component i in raw biomass)

C_{i0} = Component i content at time zero (kg Component i in raw biomass/kg raw biomass)

C_i = Component i content at time t (kg residual Component i/kg residual biomass)

Y_T = total solids pretreatment yield at time t (kg residual biomass/kg raw biomass)

Estimation of kinetic parameters

For a kinetic model to be useful, it should accurately predict the measured quantities and account for the main process variables, such as alkali concentration, temperature, and oxygen pressure. There are several models published in the literature in which mass transfer phenomena and chemical reactions are combined in a simple power law. The models account for different degradation rates that occur with lignin and carbohydrates moieties of differing reactivity including the following species: highly reactive, reactive, uncondensed, condensed, and non-reactive.[143, 144] Some of these models follow:

Single equation, high order on lignin. This model was not used in this study because it contradicts studies on the mechanisms of oxygen delignification that show first-order kinetics in residual lignin.[145] Additionally, in some instances, high-order lignin models gave abnormal reaction rates.[137]

Sum of an infinite number of parallel first-order reactions and rate constants that are interpreted as a function distribution. This model introduces large complexity to calculations, but its ability to fit the data is not considerably improved compared to simpler approaches; thus, this model was not used here.[135, 146]

The following models were used in this study: *Model 1: Two parallel, first-order reactions* and *Model 2*: *Three parallel, first-order reactions*. To accurately represent delignification and carbohydrate degradation processes with this approach, it is necessary to consider differing reactivities.[137, 138, 147] For each biomass component (lignin, cellulose, and hemicellulose), Model 1 uses two parallel simultaneous reactions

(fast and slow), illustrated in Figure 32 for lignin. This model successfully represents literature data (Table 14). Similarly, Model 2 uses three parallel simultaneous reactions (fast, medium, and slow), which are often considered in Kraft delignification.[138, 148, 149] Additionally, Models 1 and 2 use first-order kinetics on lignin because previous studies have reported evidence for this reaction order.[139, 145, 150]

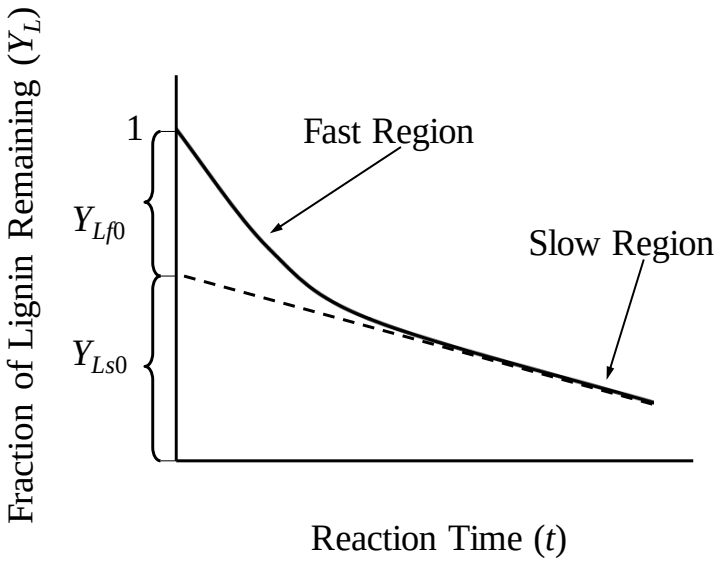

Figure 32. Two lignin moieties give rise to two simultaneous reactions according to reactivity: fast (Y_{Lf0}) and slow (Y_{Lf0}).

For lignin degradation, kinetic parameters are often obtained using Kappa number, a widely used estimate of lignin content. (*Note*: Kappa number is 6.7 times larger than the lignin content.[8]) Instead, here we use lignin yield, which was also used in kinetic modeling of lime pretreatment by Kim and Holtzapple (2006).[147, 151] The generalized Eq. 3 defines lignin, glucan, and xylan yields.

Table 14. Activation energies for oxygen delignification of pulp using two-moiety models

#	Study	Lignin moieties	Activation energy (kJ/mol)
1	Olm et al., 1979[26]	Fast	10.0
		Slow	45.0
2	Myers et al., 1989[10]	Fast	31.6
		Slow	61.4
3	Vincent et al., 1994[29]	Fast	24.2
		Slow	46.3
4	Iribane et al., 1997[9]	Fast	67.0
		Slow	40.0
5	Kim et al., 2006[30]	Fast	50.1
		Slow	54.2
6	This study	Fast	113
		Slow	44.6

Models 1 and 2 describe each biomass component (lignin, glucan, and xylan) as the sum of fast *f*, medium *m* (for Model 2 only), and slow *s* moieties

$$Y_i = \sum_j Y_{ij} \tag{4}$$

where

i = L for lignin, G for glucan, and X for xylan

j = f and s (Model 1) and f, m, and s (Model 2)

Y_{ij} = yield of Component i at time t (kg residual Component i/kg initial Component i)

At time zero,

$$Y_{i0} = \sum_j Y_{ij0} = 1 \tag{5}$$

Because an excess of lime is employed in all experiments, and lime is sparingly soluble, hydroxide concentration [OH^-] is always constant, i.e., it is not a variable in the models. As a result, the models must only describe the effects of oxygen pressure, time, temperature, and the amount of Component *i* in the residual biomass

$$-\frac{dY_i}{dt} = \sum_j k_{ij} P_{O_2}{}^{\beta ij} Y_{ij} \tag{6}$$

where

$$k_{ij} = a_{ij} \exp\left(-\frac{E_{ij}}{RT}\right) \tag{7}$$

and

k_{ij} = rate constant $((\text{min}\cdot\text{bar}^{\beta ij})^{-1})$

a_{ij} = frequency factor $((\text{min}\cdot\text{bar}^{\beta ij})^{-1})$

E_{ij} = activation energy (kJ/mol)

R = ideal gas constant (8.314×10^{-3} kJ/(mol·K))

T = absolute temperature (K)

P_{O2} = oxygen pressure (bar, absolute)

β_{ij} = exponent (dimensionless)

The integral form of Eq. 6 is

$$Y_i = \sum_j Y_{ij0} \exp(-k_{ij} P_{O2}{}^{\beta ij} t) \tag{8}$$

where Y_{ij0} is the yield of Component *ij* at time zero (kg residual Component *ij*/kg initial Component *i*)

The models parameters were obtained by minimizing the residual sum of squares calculated as $R = \sum (y - \hat{y})^2$ where y is the observed data and $\hat{y}$ the model estimate using the Levenberg-Marquardt[152] technique (LM) as implemented in Matlab R-12® (*lsqnonlin* subroutine). Because many local minima were found, various other more powerful numerical methods were tested including: Simulated Annealing (SA),[153] Interior Point methods (IP),[154] the Greedy Algorithm (G),[155] and several combinations of these methods. To solve this particular problem, stochastic methods (SA and G) proved to be less efficient than deterministic methods (IP and LM). IP gave the best results and the parameters obtained using this method are reported here. Because parameter search was extensive, there is a good chance that the reported parameters are near the global minimum of the objective function. Detailed description of parameter search techniques can be found elsewhere.[156]

Results and discussion

Oxidative short-term lime pretreatment at constant total pressure (CP) modifies poplar wood composition mainly by degrading lignin and hemicellulose; however, some cellulose is also degraded. The degradation of lignin and carbohydrates is a direct function of pretreatment temperature, total pressure, and time.[111]

Both Models 1 and 2 resulted in good data fit as shown in Figures 33, 34, 35 for lignin, glucan, and xylan, respectively. Model assessment and comparisons were performed on the basis of the highest F_c as proposed by Froment and Bischof:[157]

$$F_c = \frac{\sum_{i=1}^{n} \frac{\hat{y}_i^{\,2}}{p}}{\sum_{i-1}^{n} \frac{(y_i - \hat{y}_i)^2}{n-p}} \tag{9}$$

where

$\hat{y}_i$ = estimated value of dependent value

p = number of parameters in the model

n = number of experiments

y_i = measured data

In both Models 1 and 2 for lignin, glucan, and xylan, R was very low and F_c was very high (Table 15). Furthermore, for the predicted variables (lignin and carbohydrate yields), 95% confidence interval half-widths were very low (see footnotes in Tables 16, 18 and 19). However, parameter confidence intervals were very wide, particularly for frequency factors; which for most cases, were wider in Model 2 than in Model 1. Furthermore, for each case (lignin, glucan, and xylan), F_c for Model 2 was lower than for Model 1 because F_c rewards models for parsimony (i.e. simplicity and fewer parameters). Also, Y_{if0} was always very low for Model 2 indicating that the contribution of this term to the total sum (Eq. 8) was small. Based on this analysis, Model 1 was chosen over Model 2 and the results obtained with this model are discussed next.

Table 15. F_c and sum of squared residuals for Models 1 and 2

	F_c		Sum of squared residuals	
Model	Two moieties (Model 1)	Three moieties (Model 2)	Two moieties (Model 1)	Three moieties (Model 2)
Lignin	630	520	0.233	0.166
Glucan	8830	2760	0.026	0.051
Xylan	1000	570	0.181	0.199

Lignin degradation. Figure 33 shows that Model 1 overestimates delignification at low temperature (110°C) and at pretreatment times >400 min. At temperatures of 140°C and pressures above 14.8 bars, Model 1 underestimates delignification. At temperatures of 160°C and 180°C, the model fits the experimental data very well. Observe that at 180°C and 7.9 bars, a very poor delignification response is achieved. This is explained because the partial pressure of steam is very high and displaces oxygen.

According to Model 1, the average yield of fast-lignin at time zero (Y_{Lf0}) is 0.384 g lignin/g lignin in raw biomass (Figure 33 and Table 16). The Y_{Lf0} closest to the average corresponds to 140°C and 21.7 bar. Higher and lower Y_{Lf0} are observed for higher or lower temperatures and pressures, respectively.

Changes of slope from high (mostly fast-degrading lignin) to low (mostly slow-degrading lignin) were observed between ~150 min (at 180°C) and ~250 min (at 110°C). Because Y_{Lf0} is related to the fraction of easy-to-degrade lignin, these results indicate a strong effect of temperature and pressure on the rate of lignin degradation.

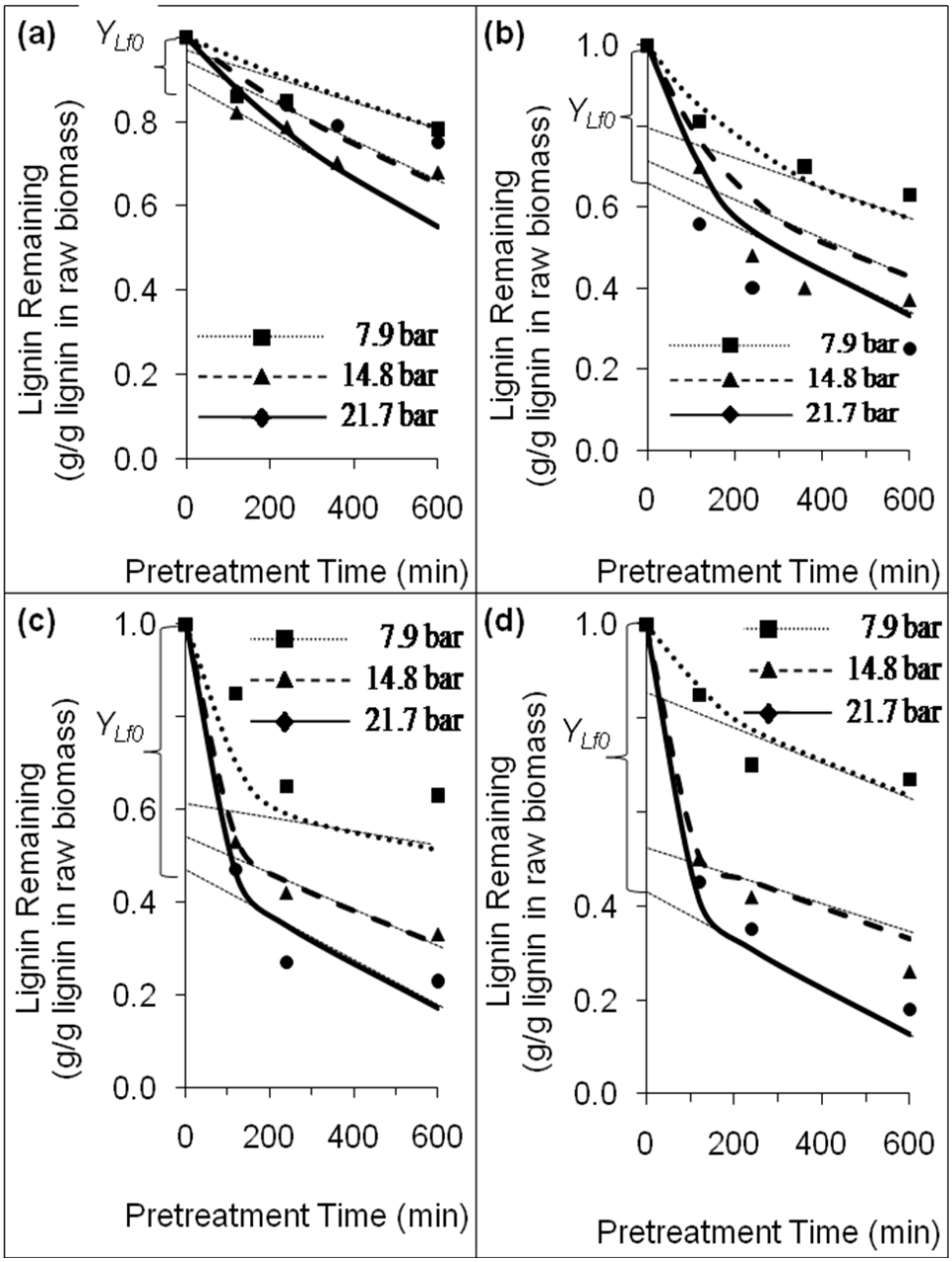

Figure 33. Data fit for lignin degradation using Model 1 (a) 110°C (b) 140°C (c) 160°C and (d) 180°C.

Table 16. Parameter estimates and confidence intervals for lignin using Models 1 and 2 ($\alpha = 0.05$)

Parameter	Units	Parameter ± confidence interval	
		Model 1[a]	Model 2[b]
Y_{Lf0}	$\frac{\text{g lignin remaining}}{\text{g lignin in raw biomass}}$	0.384 ± 0.097	0.117 ± 0.082
a_{Lf}	min^{-1}	$5.97\times10^{11} \pm 8.73\times10^{12}$	0.0129 ± 0.751
E_{Lf}	kJ/mol	113 ± 50.2	47.5 ± 122
β_{Lf}	dimensionless	0.714 ± 0.336	8.04 ± 14.6
Y_{Lm0}	$\frac{\text{g lignin remaining}}{\text{g lignin in raw biomass}}$	–	0.358 ± 0.098
a_{Lm}	min^{-1}	–	$5.90\times10^{11} \pm 9.63\times10^{12}$
E_{Lm}	kJ/mol	–	113 ± 56.9
β_{Lm}	dimensionless	–	0.631 ± 0.310
Y_{Ls0}	$\frac{\text{g lignin remaining}}{\text{g lignin in raw biomass}}$	$0.616 \pm \text{NA}$[c]	$0.526 \pm \text{NA}$[d]
a_{Ls}	min^{-1}	30.5 ± 173	59.1 ± 497
E_{Ls}	kJ/mol	44.6 ± 21.3	47.9 ± 30.4
β_{Ls}	dimensionless	1.05 ± 0.578	1.02 ± 0.74

[a] 95% confidence interval half-widths for the predicted variable (Y_L) varied between 0 and 0.016
[b] 95% confidence interval half-widths for the predicted variable (Y_L) varied between 0 and 1.19
[c] Calculated as $1-Y_{Lf0}$
[d] Calculated as $1- Y_{Lf0}- Y_{Lm0}$

Temperature affects the internal energy available for reaction whereas oxygen pressure relates to solubility, which affects both reaction kinetics and diffusion.[158] At 140°C, 21.7 bars, and 600 min, complete degradation of Y_{Lf0} fraction was observed. If T >140°C, lower pressures and times were required for complete degradation of Y_{Lf0}. In this fast stage of delignification, electrophilic attack of lignin and peeling at the hemicellulose-lignin bonds occurs. At the most severe pretreatment conditions (180°C, 21.7 bars, and 600 min), 17% of Y_{Ls0} still remained. This inability to completely degrade lignin may be explained by some condensation reactions that may have occurred leaving lignin inert.

The rate constant for k_{Lf} is 9 to 240 times greater than the corresponding k_{Ls} with the greatest differences at the highest temperatures. Activation energies for slow and fast moieties (45 and 113 kJ/mol, respectively) have a similar order of magnitude to those in other studies (Table 14); however, E_{Ls} is low compared to typical values in most chemical reactions. This implies that this stage is diffusing controlled rather than chemically controlled.

The oxygen reaction order for fast-degrading lignin (β_{Lf}) was ~2/3 the oxygen reaction order for slow-degrading lignin (β_{Ls}), which was close to 1.0 (Table 16). This implies that oxygen is important in both stages of delignification and may explain why diffusion is rate controlling.

Glucan degradation. In pulp bleaching, the cleavage of polysaccharide chains is usually monitored by the number-average moles of cellulose per metric ton of pulp (m_n).

In lime pretreatment, the variable of interest is glucan yield (Y_G) defined in Eq. 4. Interestingly, oxygen bleaching of pulp uses zero-order kinetics on glucan (Table 17). Olm and Teder[147] explained this phenomena by considering that the total number of carbohydrate bonds does not decrease substantially as the reactions proceed for two main reasons: (1) the pulp has previously been subjected to a severe alkaline environment in the digester where the stopping reaction gives the cellulose a high content of stable end groups, and (2) oxygen itself converts reducing end groups to stable oxidized forms.[101] In this study, these conditions do not necessarily occur, and first-order kinetics for glucan yields are considered.

Table 17. Kinetic parameters for polysaccharide cleavage of pulp during oxygen delignification as reported by widely cited studies

Study	Cellulose moieties	Activation energy (kJ/mol)	m_n[a] Reaction order
Olm et al., 1979[26]	Fast	40	0
	Slow	53	0
Iribane et al., 1997[9]	Only one	78	0

[a] m_n is the number-average moles of cellulose per metric ton of pulp.

Figure 34 shows that higher temperatures, pressures, and longer pretreatment times result in a much higher glucan degradation up to a minimum of 0.48 g glucan remaining/g glucan in raw biomass observed at 180°C, 21.7 bar, and 600 min.

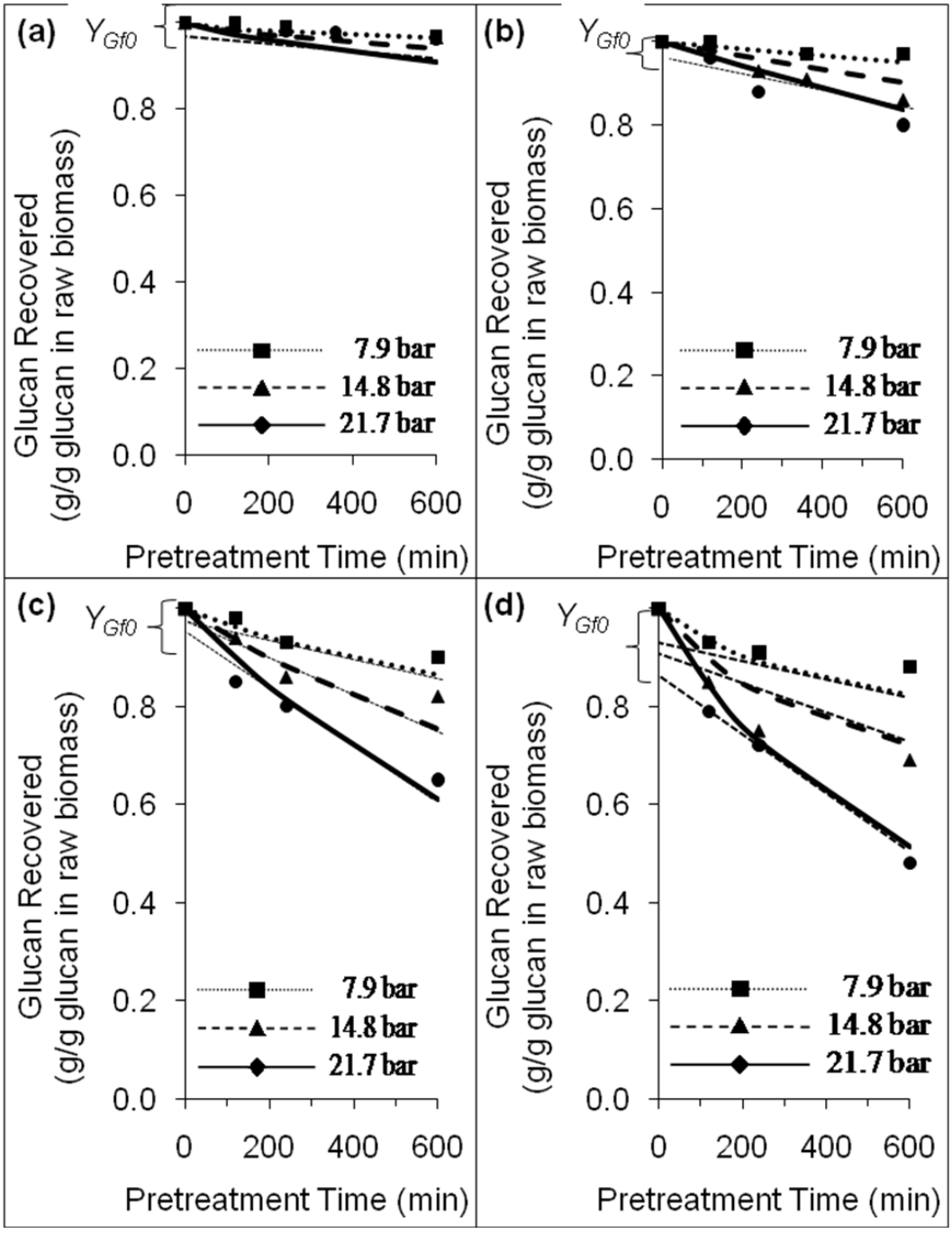

Figure 34. Data fit for glucan degradation using Model 1 (a) 110°C (b) 140°C (c) 160°C and (d)180°C.

Table 18. Parameter estimates and confidence intervals for glucan using Models 1 and 2 ($\alpha = 0.05$)

Parameter	Units	Parameter ± confidence interval	
		Model 1[a]	Model 2[b]
Y_{Gf0}	$\frac{\text{g glucan remaining}}{\text{g glucan in raw biomass}}$	0.110 ± 0.0775	$0.0104 \pm$ NA [d]
a_{Gf}	min^{-1}	$5.13\times10^{6} \pm 4.02\times10^{7}$	$10.0 \pm 1.94\times10^{22}$
E_{Gf}	kJ/mol	75.0 ± 33.4	$0.499 \pm 3.58\times10^{22}$
β_{Gf}	dimensionless	0.249 ± 0.153	$0.500 \pm 3.46\times10^{22}$
Y_{Gm0}	$\frac{\text{g glucan remaining}}{\text{g glucan in raw biomass}}$	–	0.306 ± 0.231
a_{Gm}	min^{-1}	–	1773 ± 94264
E_{Gm}	kJ/mol	–	66.8 ± 25.9
β_{Gm}	dimensionless	–	1.88 ± 1.26
Y_{Gs0}	$\frac{\text{g glucan remaining}}{\text{g glucan in raw biomass}}$	$0.890 \pm$ NA [c]	0.684 ± 0.233
A_{Gs}	min^{-1}	$5.95\times10^{4} \pm 123$	$2.03\times10^{3} \pm 3.48\times10^{9}$
E_{Gs}	kJ/mol	77.6 ± 19.9	56.3 ± 65.1
β_{Gs}	dimensionless	1.11 ± 0.763	$1.10\times10^{-11} \pm 0.218$

[a] 95% confidence interval half-widths for the predicted variable (Y_G) varied between 0 and 0.124
[b] 95% confidence interval half-widths for the predicted variable (Y_G) varied between 0 and 0.0839
[c] Calculated as $1-Y_{Gf0}$
[d] Calculated as $1- Y_{Gm0}- Y_{Gs0}$

According to Model 1, the average yield of fast-degrading glucan at time zero (Y_{Gf0}) for all temperatures and pressures is 0.110 g glucan/g glucan in raw biomass (Figure 34, Table 18); thus, $Y_{Lf0} > Y_{Gf0}$ implying that lignin degrades much faster than cellulose in the alkaline oxidative media of short-term-CP lime pretreatment. Similar to lignin degradation, glucan degradation is triggered by high temperatures (i.e., higher internal energy) and high pressures (i.e., higher oxygen solubility), which results in higher Y_{Gf0}. The change from high to low slope occurs between ~200 min (180°C) and ~380 min (110°C), which is after lignin changes occur (see Section 5.1); thus, cellulose and lignin degradations are not related to each other in oxidative alkaline media.

At 160°C, 600 min, and 21.7 bars, Y_{Gf0} completely degraded. If T = 180°C, less pressure and time were required for complete Y_{Gf0} degradation. At the most severe pretreatment conditions (180°C, 600 min, and 21.7 bars), 50% of Y_{Gs0} still remained; thus, short-term-CP lime pretreatment is more aggressive on lignin than it is on glucan.

The rate constant for k_{Gf} is 170 to 200 times greater than the corresponding k_{Gs} with the greatest differences at the lowest temperatures. Activation energies for fast and slow moieties are 75 and 77 kJ/mol, respectively (Table 18), which is similar to those reported by other studies (Table 17). They are low compared to typical values in chemical reactions; thus, glucan reaction mechanisms are diffusion rather than chemically controlled.

Unlike lignin degradation, the oxygen reaction order for fast-degrading glucan (β_{Gf}) was small. It was only ~1/3 the oxygen reaction order for slow-degrading lignin, which was $\beta_{Gs} \approx 1.0$ (Table 18). Consequently, oxygen is important in the second

delignification stage. As stated in the previous paragraph, diffusion is rate controlling at the beginning of pretreatment, likely because of hard-to-reach cellulose, rather than to oxygen unavailability.

Xylan degradation. Kinetic modeling of hemicellulose degradation is important because the contribution of this carbohydrate polymer to the total carbohydrate yield is potentially significant. For Model 1, the parameters with their corresponding confidence intervals are shown in Table 19 and data fit is shown in Figure 35. For most conditions tested, a fair representation of the data is obtained with Model 1 but an important overestimation of xylan degradation is observed for 160°C and 7.9 bar at all pretreatment times and at 180°C, 21.7 bar, and 600 min.

According to Model 1, for all temperatures and pressures, the average yield of fast-degrading xylan at time zero (Y_{Xf0}) is 0.365 g xylan/g xylan in raw biomass (Table 19); thus, $Y_{Lf0} \approx Y_{Xf0}$. Similar to lignin and cellulose degradation, xylan degradation is triggered by higher temperatures and pressures. Furthermore, in xylan degradation curves, the change from high to low slope occurs at the same times as in lignin degradation (i.e., between ~150 min (180°C) and ~250 min (110°C). Xylan degradation is much more significant than glucan degradation and behaves very similar to lignin degradation. This is because of covalent bonds between hemicellulose and lignin in the cell wall.[125] At 160°C, 600 min, and 7.9 bars, Y_{Xf0} completely degraded.

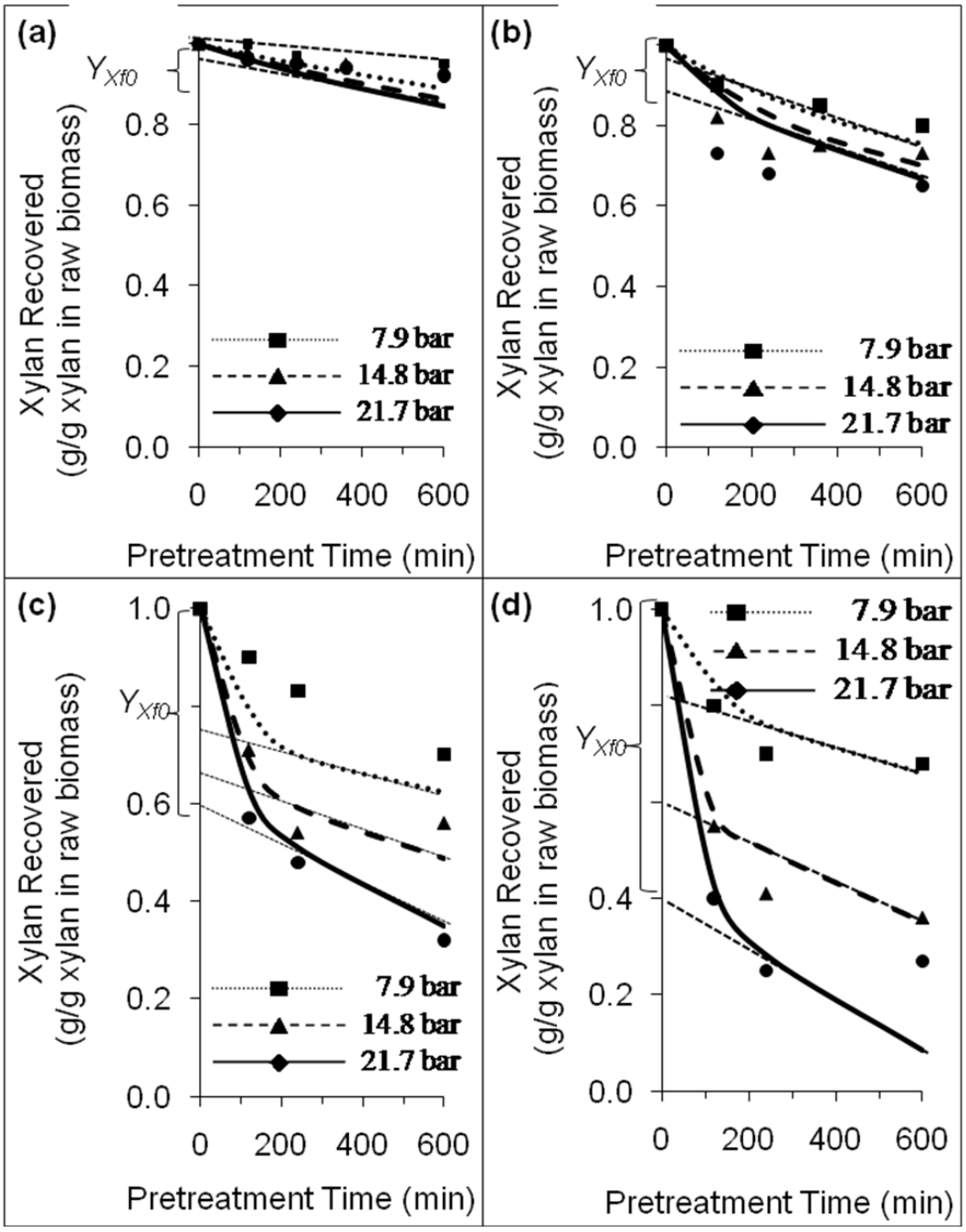

Figure 35. Data fit for xylan degradation using Model 1 (a) 110°C (b) 140°C (c) 160°C and (d) 180°C.

Table 19. Parameter estimates and confidence intervals for xylan using Models 1 and 2 ($\alpha = 0.05$)

Parameter	Units	Parameter ± confidence interval	
		Model 1[a]	Model 2[b]
Y_{Xf0}	$\frac{\text{g xylan remaining}}{\text{g xylan in raw biomass}}$	0.365 ± 0.115	0.0586 ± 0.0834
a_{Xf}	min^{-1}	$2.70\times10^{7} \pm 1.97\times10^{8}$	871 ± 51708
E_{Xf}	kJ/mol	80.3 ± 24.8	66.3 ± 265
β_{Xf}	Dimensionless	0.355 ± 0.185	3.78 ± 13.7
Y_{Xm0}	$\frac{\text{g xylan remaining}}{\text{g xylan in raw biomass}}$	–	0.545 ± 0.152
a_{Xm}	min^{-1}	–	$4.20\times10^{6} \pm 3.40\times10^{7}$
E_{Xm}	kJ/mol	–	76.9 ± 29.0
β_{Xm}	Dimensionless	–	0.315 ± 0.131
Y_{Xs0}	$\frac{\text{g xylan remaining}}{\text{g xylan in raw biomass}}$	0.635 ± NA [c]	0.397 ± NA [d]
a_{Xs}	min^{-1}	$1.87\times10^{11} \pm 2.71\times10^{12}$	354 ± 11915
E_{Xs}	kJ/mol	132 ± 58.1	88.8 ± 239
β_{Xs}	Dimensionless	1.38 ± 0.770	4.25 ± 13.6

[a] 95% confidence interval half-widths for the predicted variable (Y_X) varied between 0 and 0.0990
[b] 95% confidence interval half-widths for the predicted variable (Y_X) varied between 0 and 0.773
[c] This parameter was calculated as $1-Y_{Xf0}$
[d] This parameter was calculated as $1- Y_{Xf0}- Y_{Xm0}$

If T = 180°C, less pressure and time were required for complete Y_{Xf0} degradation Interestingly, this puts complete degradation of fast xylan fraction somewhere in between complete degradation of fast lignin fraction and fast glucan fraction. At the most severe pretreatment conditions (180°C, 600 min, and 21.7 bars), 13% of Y_{Xs0} still remained. Because this is less than maximum lignin degradation, short-term-CP lime pretreatment is more damaging to xylan than to lignin.

The rate constant k_{Gf} is 130 to 1600 times greater than the corresponding k_{Gs} with the greatest differences at the lowest temperatures. The activation energy for fast xylan is 80 kJ/mol (Table 19), which is a little higher than activation energies for fast lignin and glucan. Similar to these cases, fast-xylan degradation is diffusion controlled rather than chemically controlled. Interestingly, the activation energy for slow xylan is much higher (132 kJ/mol, see Table 19), which indicates that chemical control may be as important as diffusion in this case. Similar to xylan degradation, the oxygen reaction order for fast-degrading xylan (β_{Xf}) was only a fraction of the oxygen reaction order for slow-degrading xylan (β_{Xs}), which was similar to both slow-lignin and slow-glucan degradation (~1.0) (Table 19). However, unlike both lignin and glucan degradation, activation energies for xylan are high indicating that diffusion is not controlling. Consequently, oxygen radicals are likely to attack xylan (most probably at the xylan-lignin bonds) before they reach lignin and glucan.

Model assessment

According to the discussions above, Model 1 adequately describes lignin, glucan, and xylan degradation. This model was compared to the experimental data through cumulative mass profiles of all three components of interest (lignin, glucan and xylan) against time. Good agreement was found (a few examples are shown in Figure 36). Additionally, most data fit the model within 10% (Figure 37).

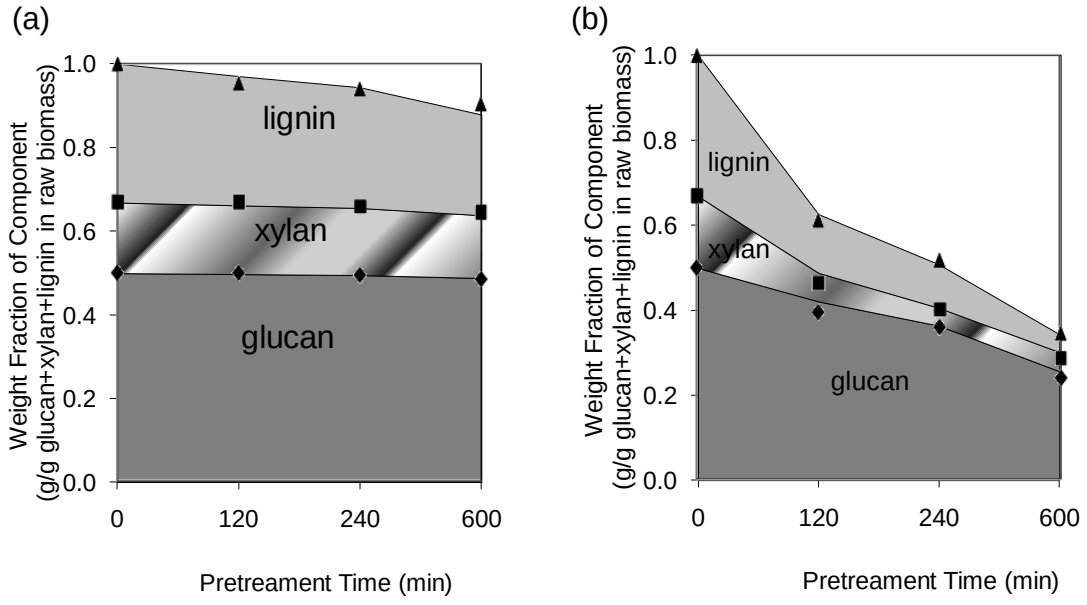

Figure 36. Experimental (data points) and model estimated (continuous lines) mass profiles for glucan, lignin, and xylan at (a) 110°C and 7.9 bar (b) 180°C and 21.7 bar.

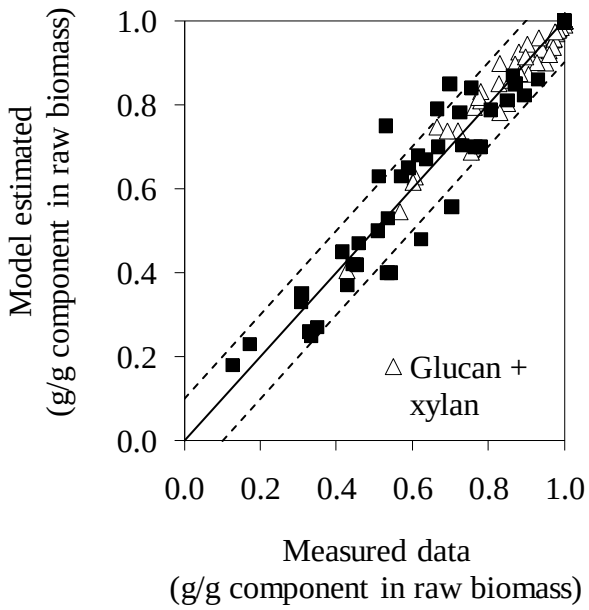

Figure 37. Model assessment. Dotted lines describe 95% prediction intervals.

Selectivity

An important feature of pretreatment is its ability to selectively remove lignin without extensive attack on carbohydrate fractions. According to previous studies on oxygen bleaching selectivity, hydroxyl radical (HO·) degrades nonphenolic lignin compounds five to six times faster than carbohydrate compounds;[159] however, because of the great variability of active oxygen species produced in oxygen bleaching, this conclusion cannot be generalized. Furthermore, lignin reactivity is governed by the structure of non-phenolic lignin and is proportional to the number of hydroxyl groups in carbohydrates and lignin.[160]

Differential selectivity is the ratio of lignin degradation rate to carbohydrate degradation rate. Ideally the selectivity should be high. Using Model 1, the mathematical expressions for this selectivity follow:

$$S_{dG} \equiv \frac{dY_L/dt}{dY_G/dt} = \frac{a_{Lf}\exp\left(-\frac{E_{Lf}}{RT}\right)P_{O2}^{\beta Lf}Y_{Lf} + a_{Ls}\exp\left(-\frac{E_{Ls}}{RT}\right)P_{O2}^{\beta Ls}Y_{Ls}}{a_{Gf}\exp\left(-\frac{E_{Gf}}{RT}\right)P_{O2}{}^{\beta Gf}Y_{Gf} + a_{Gs}\exp\left(-\frac{E_{Gs}}{RT}\right)P_{O2}{}^{\beta Gs}Y_{Gs}}$$

(10)

$$S_{dX} \equiv \frac{dY_L/dt}{dY_X/dt} = \frac{a_{Lf}\exp\left(-\frac{E_{Lf}}{RT}\right)P_{O2}^{\beta Lf}Y_{Lf} + a_{Ls}\exp\left(-\frac{E_{Ls}}{RT}\right)P_{O2}^{\beta Ls}Y_{Ls}}{a_{Xf}\exp\left(-\frac{E_{Xf}}{RT}\right)P_{O2}^{\beta Xf}Y_{Xf} + a_{Xs}\exp\left(-\frac{E_{Xs}}{RT}\right)P_{O2}^{\beta Xs}Y_{Xs}}$$

(11)

With oxidative lime pretreatment, glucan selectivity is ~10 times higher than xylan selectivity. The best selectivities were observed for high lignin content, high temperature, low pressure, and the initial phase of pretreatment (Figure 38). Although glucan and xylan selectivities decrease as lignin content reduces and as time passes, the effect is more noticeable in glucan selectivity.

For residual lignin >0.5 kg lignin remaining/kg lignin in raw biomass, glucan selectivity is higher than xylan selectivity. For instance, at the beginning of pretreatment, 180°C, and 14.8 bars, S_{dG} is ~10 times higher than S_{dX}. However, as pretreatment occurs, S_{dG} decreases faster than S_{dX}. At the temperature and pressure listed above, for residual

lignin ≤0.5 kg lignin remaining/kg lignin in raw biomass, glucan and xylan selectivities become similar. The dependence of xylan selectivity on pressure, temperature, and pretreatment time is smaller than that of glucan. The minimum selectivity observed was about 1 g lignin removed/g glucan or xylan removed.

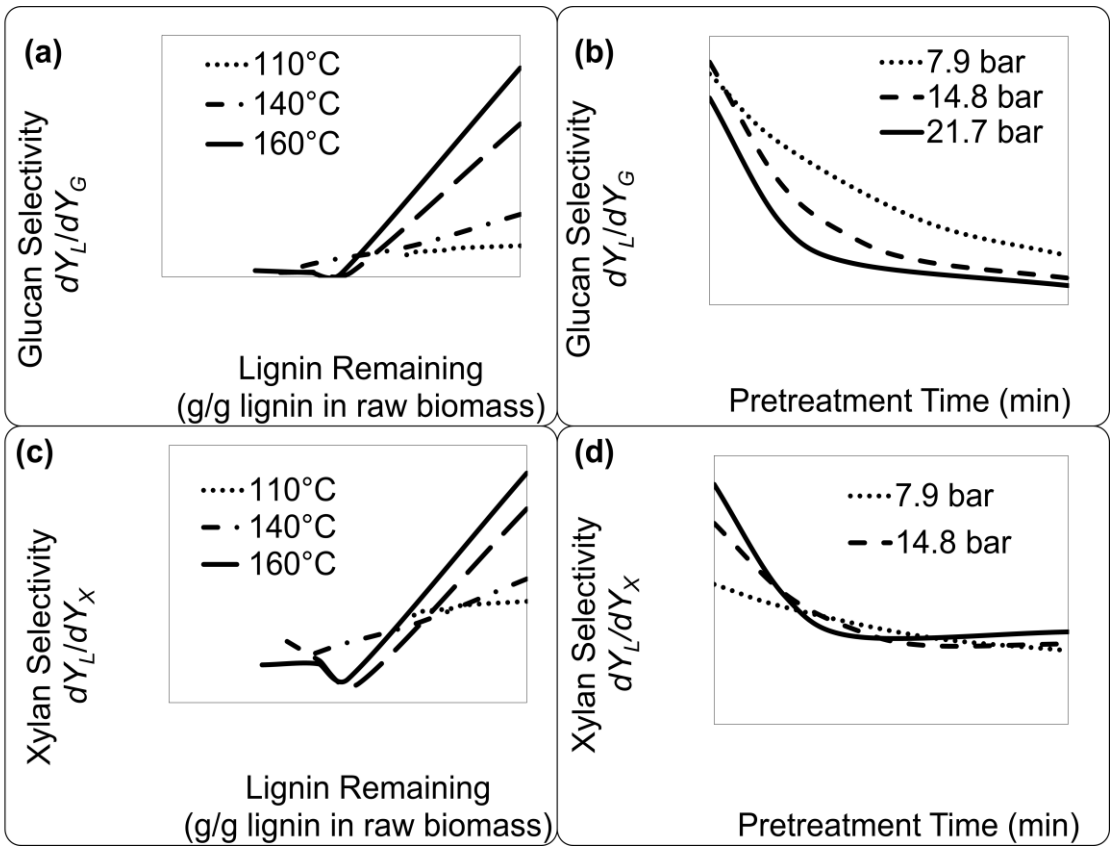

Figure 38. Differential selectivity (a) for glucan at 14.8 bar total pressure (b) for glucan at 140°C (c) for xylan at 14.8 bar total pressure (d) for xylan at 140°C.

Integral selectivity is the ratio of lignin removed to the amount of carbohydrates removed at a particular time during pretreatment. Mathematical expressions for these selectivities follow:

$$S_G \equiv \frac{1-Y_L}{1-Y_G} = \frac{1-Y_{Lf0}\exp\left(-a_{Lf}\exp\left(-\frac{E_{Lf}}{RT}\right)P_{O2}^{\beta Lf}t\right)-Y_{Ls0}\exp\left(-a_{Ls}\exp\left(-\frac{E_{Ls}}{RT}\right)P_{O2}^{\beta Ls}t\right)}{1-Y_{Gf0}\exp\left(-a_{Gf}\exp\left(-\frac{E_{Gf}}{RT}\right)P_{O2}^{\beta Gf}t\right)-Y_{Gs0}\exp\left(-a_{Gs}\exp\left(-\frac{E_{Gs}}{RT}\right)P_{O2}^{\beta Gs}t\right)}$$

(12)

$$S_X \equiv \frac{1-Y_L}{1-Y_X} = \frac{1-Y_{Lf0}\exp\left(-a_{Lf}\exp\left(-\frac{E_{Lf}}{RT}\right)P_{O2}^{\beta Lf}t\right)-Y_{Ls0}\exp\left(-a_{Ls}\exp\left(-\frac{E_{Ls}}{RT}\right)P_{O2}^{\beta Ls}t\right)}{1-Y_{Xf0}\exp\left(-a_{Xf}\exp\left(-\frac{E_{Xf}}{RT}\right)P_{O2}^{\beta Xf}t\right)-Y_{Xs0}\exp\left(-a_{Xs}\exp\left(-\frac{E_{Xs}}{RT}\right)P_{O2}^{\beta Xs}t\right)}$$

(13)

At $t = 0$, $Y_L = Y_G = Y_X = 1$, consequently S_G and S_X are indeterminate; however, this issue is easily solved given that

$$\lim_{t\to 0}\left(1-Y_L\right) = \lim_{t\to 0}\left(1-Y_i\right) = 0$$

(14)

where $i =$ *G* for glucan and *X* for xylan, and

$$\lim_{t\to 0}\left(\frac{\frac{d}{dt}\left(1-Y_L\right)}{\frac{d}{dt}\left(1-Y_i\right)}\right) = \lim_{t\to 0}\left(\frac{\frac{d}{dt}\left(\varepsilon_L\right)}{\frac{d}{dt}\left(\varepsilon_i\right)}\right) = \lim_{t\to 0}\left(\varepsilon_{di}\right) = S_{di} \qquad (15)$$

By applying L'Hôpital's rule at $t = 0$

$$S_i = \lim_{t\to 0}\left(\frac{1-Y_L}{1-Y_i}\right) = \lim_{t\to 0}\left(\frac{\frac{dY_L}{dt}}{\frac{dY_i}{dt}}\right) = S_{di} \qquad (16)$$

Thus, at $t = 0$, the value already calculated for S_{di} can be used for S_i.

In general, for most pretreatment conditions tested, both S_G and S_X have numerical values that are very similar to the corresponding S_{dG} and S_{dX}. This is particularly true at low temperatures and pressures and at the beginning of pretreatment. However, for high lignin content, there are some cases (temperature ≥140°C, pressure ≥14.8 bar, and lignin content ≤0.5 g lignin remaining/g lignin in raw biomass) where integral selectivity (especially integral glucan selectivity) is 2 to 3 times greater than the corresponding differential selectivity.

Integral selectivity is particularly useful because it determines the relative yields at a particular point in the reaction, which is valuable for design purposes.

Conclusions

Lime pretreatment improves lignocellulosic biomass digestibility by selectively removing lignin while retaining carbohydrates. Through models developed in this study, the best pretreatment conditions can be identified, leading to optimal results.

Kinetic models traditionally used in oxygen bleaching delignification of pulps consider two or three different lignin moieties (rapid, medium, and slow). These models were successfully applied to oxidative lime pretreatment. Additionally, the pretreatment models described glucan and xylan degradation as well. For lignin, glucan, and xylan, on

the basis of best fit and statistical analysis, Model 1 (two moieties) was preferred to Model 2 (three moieties).

For delignification, all obtained activation energies for delignification ranged from 45 to113 kJ/mol. This is similar to previous kinetic studies on biomass delignification through oxidative-lime pretreatment, which reported activation energies close to 50 kJ/mol.[151] The activation energies for glucan degradation ranged from 45 to 80 kJ/mol, except for E_{Gs} in Model 2, which was only 0.5 kJ/mol. The activation energies for xylan degradation were higher, ranging between 65 and 132 kJ/mol.

These models were used to calculate glucan and xylan selectivity in two fashions: differential and integral. These two definitions gave similar values particularly for low temperatures, pressures, and pretreatment times. The highest glucan selectivity was about 17 g lignin removed/g glucan removed, and the highest xylan selectivity was about 3.8 g lignin removed/g xylan removed; the former was observed at 0 min, 7.9 bar, and 140°C, and the later at 0 min, 7.9 bar, and 110°C.

Both glucan and xylan integral and diferential selectivities are higher at lower temperatures and pressures, and at the beginning of pretreatment (i.e., for high lignin content). The rate of change of glucan selectivity decreases smoothly with pretreatment time at temperatures ≤140°C. At higher temperatures, glucan selectivity decreases quickly with pretreatment time until 200 min. For longer pretreatments, glucan selectivity remains almost constant but is less than 4 g lignin removed/g glucan or xylan removed.

Xylan selectivity changes more slowly with pretreatment time than glucan selectivity. Effects of temperature, pretreatment time, and pressure exist, but are not as significant as with glucan selectivity.

At high temperatures and low lignin content (<0.5 g lignin remaining/g lignin in raw biomass), the detrimental effect of pretreatment on selectivity is considerable, as has been amply discussed in the literature.[143, 145, 147]

SELECTIVITY AND DELIGNIFICATION KINETICS FOR OXIDATIVE SHORT-TERM LIME PRETREATMENT OF POPLAR WOOD. PART II: VARYING-PRESSURE

Synopsis

Lime pretreatment improves lignocellulosic biomass digestibility by removing lignin. Through kinetic models, the best pretreatment conditions can be identified that selectively remove lignin while preserving carbohydrates. Models traditionally used in oxygen bleaching delignification of pulps are successful when applied to lime pretreatment. This study focuses on obtaining model parameters to fit experimental data for poplar wood lime pretreated at the following conditions: time 1 to 10 hours, temperature 140 to 180°C, total initial partial pressure of oxygen 7.9 to 28.6 bar, and excess lime loading of 0.5 g $Ca(OH)_2$/g dry biomass. The models properly fit experimental data and were used to determine pretreatment selectivity, which assesses the detrimental effect of pretreatment on carbohydrate yield. Selectivity was defined in two ways: differential and integral. The models can be used to identify pretreatment conditions that selectively remove lignin while preserving carbohydrates. Lignin removal ≥50% with glucan preservation ≥90% was observed for differential glucan selectivity between 40 and 70 g lignin degraded/g glucan degraded. This combination was observed at 140°C, pressures ≥14.7 bar, and times between 6 and 10 hours. For 160°C, a much higher lignin removal (~70%) with glucan preservation ≥90% was observed at 6 hours and all pressures. The corresponding differential glucan selectivities

ranged between ~3 and ~4.5 g lignin degraded/g glucan degraded. Other pretreatment conditions resulted in either too little lignin degradation or too much carbohydrate degradation.

Introduction

Oxygen pulping and bleaching of wood is analogous to lime pretreatment of biomass because of similar conditions (alkaline and oxidative) and end purposes (delignification and carbohydrate preservation); however, the ultimate goals differ. Pulping and bleaching seek complete delignification, whereas biomass complete delignification is not required to make the biomass fully digestible.[24, 161] In fact, it is not necessary to go below ~12% lignin content to achieve good enzymatic digestibility.[24] Lime pretreatment prepares biomass for fermentation or animal feed by increasing its digestibility through structural changes, mainly lignin degradation. However, oxygen delignification is accompanied by undesirable carbohydrate degradation. Control and optimization of industrial chemical processes often require kinetic models to help determine pretreatment conditions that selectively remove lignin while preserving carbohydrates. This requires kinetic models for both lignin and carbohydrate degradation. The mechanisms of all of these reactions have been amply discussed in the literature.[101, 106, 137]

Most wood pulping kinetic models are formulated empirically using a standard power law rate equation with two or three different lignin or carbohydrate moieties considered (fast, medium, and slow).[139, 147]

This article is part of a four-paper series that summarizes the results of kinetic modeling of oxidative lime pretreatment of poplar wood with the following topics: (I) constant-pressure pretreatment, in which oxygen is replenished as it is consumed;[162] (II) varying-pressure pretreatment (this paper), in which oxygen is loaded at the beginning of pretreatment only; (III) low-temperature and long-term pretreatment;[126] and (IV) comparison of different modes of lime pretreatment to recommend conditions that optimize selectivity.[112]

Oxygen bleaching is typically used after kraft pulping to remove lignin before more expensive chemicals are applied. Usually, during this stage, 50% delignification is achieved. Alkaline conditions and oxygen cause hydroxyl groups to ionize and consequently attack free phenolic hydroxyl groups. Afterwards, an electron is removed from the phenolic oxygen using molecular oxygen as an acceptor. Further delignification reactions involve the formation of several different acids (e.g., muconic) that continue to introduce hydrophilic groups into the lignin structure. Nucleophilic attack may also occur causing ring opening, which promotes further degradation and solubilization. Condensation products may leave remaining lignin unreactive in the oxidative alkaline media.[142]

The onset of cellulose degradation occurs at a relatively rapid rate, governed by peeling mechanisms that depend on the concentration of reducing end groups.[97] Unfortunately, the hydroxyl radicals that are responsible for liginin degradation, also degrade cellulose by randomly cleaving glycosidic linkages.[142] Consequently, limiting or

preventing hydroxyl radical formation is essential to hinder this stage of carbohydrate degradation during oxygen delignification.

By charging the reactor with a fixed quantity of oxygen, the concentration of solubilized oxygen in the reacting media is high at the beginning of the process. Later, this concentration diminishes as pretreatment occurs. The aim of this study is to model lignin and carbohydrate degradation, in batch pretreatment reactors where oxygen depletes with time, and thereby calculate selectivity.

Methods

Poplar wood feedstock (8 g, dry weight) was mixed with 4 g of $Ca(OH)_2$ and 120 g of water in a 145-mL batch reactor. These reactors were made using 304 stainless steel nipples that were 5-inch long (0.127 m), 1.5-inch inside diameter (0.0381 m), and were sealed at both ends using 1.5-inch 304 stainless steel caps. All experimental details have been reported elsewhere.[111] Three temperatures were tested: 140, 160, and 180°C. After closing tightly, oxygen was applied through a quick connector located on top of the caps,[111] which charged the reactor to the desired initial pressure (Table 20). The reactors were then mounted on a rotary shaft and located inside an oven preheated to the pretreatment temperature. Rotation speed was set to 10 to 30 rpm. Pretreatment conditions were held for 1 to 10 hours. Once the pretreatment time elapsed, the reactors were removed from the oven and quickly cooled in a water-ice bath.

Table 20. Initial oxygen pressure and initial oxygen loading (M_{O2})

Oxygen pressure		Initial O_2 charge (m)[(a)] (kg O_2)	Dry weight biomass charge (kg)	Initial oxygen loading (M_{O2}) (kg O_2/kg biomass)
(bar)	(kPa)			
7.91	791	8.48×10^{-4}	0.008	0.106
14.8	1480	1.59×10^{-3}	0.008	0.198
21.7	2170	2.33×10^{-3}	0.008	0.291
28.6	2860	3.06×10^{-3}	0.008	0.383

[(a)]calculated as $m = M\ P_{O2}V/RT_r$ where P_{O2} = initial oxygen partial pressure (kPa), V = free reactor volume after filled with biomass, water, and lime (8.30×10^{-5} m^3),, R = ideal gas constant (8.314 kPa·m^3/(kmol·K)), T_r = absolute room temperature (298 K), M = oxygen molecular weight (32 kg/kmol)

When the reactors were placed in the oven, the temperature and therefore the total pressure increased (oxygen expansion and steam formation). Later, as oxygen was consumed, the total pressure decreased. This paper describes results with this varying total pressure.

Lignin and carbohydrate measurements were performed according to National Renewable Energy Laboratory Analytical Procedures.[113, 115, 116, 118] Additional details on the experimental equipment and analytical methods have been published elsewhere.[111] These measurements are reported in terms of lignin, glucan, and xylan yields defined as follows:

$$Y_i \equiv \frac{C_i \cdot Y_T}{C_{i_0}} \tag{17}$$

where

i = lignin L, glucan G, or xylan X

Y_i = pretreatment yield of Component *i* at time *t* (kg residual Component *i*/kg Component *i* in raw biomass)

C_{i0} = Component *i* content at time zero (kg Component *i* in raw biomass/kg raw biomass)

C_i = Component *i* content at time *t* (kg residual Component *i*/kg residual biomass)

Y_T = total solids pretreatment yield at time *t* (kg residual biomass/kg raw biomass)

Estimation of kinetic parameters

As discussed in Part I of this series, several kinetic models use a standard power law rate equation to describe the main effects of process variables (alkali concentration, temperature, and oxygen pressure) in oxidative, alkaline delignification of lignocellulose. Two of these models employ a single equation, high order on lignin[145] or an infinite sum of parallel first-order reactions and rate constants described by function distributions.[135, 146] Neither of these approaches were used here because they do not comply with the experimental reaction order or they are unnecessarily complex. Instead, the following models were used: *Model 1*, considers two parallel, first-order reactions for fast *f* and slow *s* lignin and *Model 2* considers three parallel, first-order reactions for fast *f*, medium *m*, and slow *s* lignin. This approach successfully represents the data.[137, 138, 147] Similar models are used to describe the degradation of glucan and xylan. Models 1 and 2 describe each component (lignin, glucan, and xylan) as the sum of fast *f*, medium *m*, and slow *s* moieties:

$$Y_i = \sum_j Y_{ij} \tag{18}$$

where

i = L for lignin, G for glucan, and X for xylan

j = f and s (Model 1) and f, m, and s (Model 2)

Y_{ij} = yield of Component i at time t (kg residual Component i/kg initial Component i)

At time zero,

$$Y_{i0} = \sum_j Y_{ij0} = 1 \tag{19}$$

Because an excess of lime is employed in all experiments and lime is sparingly soluble, hydroxide concentration [OH^-] is always constant and is not a variable in the models; therefore, Component i degradation is modeled as:

$$-\frac{dY_i}{dt} = \sum_j k_{ij} M_{O_2}{}^{\beta ij} Y_{ij} \tag{20}$$

where

$$k_{ij} = a_{ij} \exp\left(-\frac{E_{ij}}{RT}\right) \tag{21}$$

and

k_{ij} = rate constant (min^{-1})

a_{ij} = frequency factor (min^{-1})

E_{ij} = activation energy (kJ/mol)

R = ideal gas constant (8.314×10^{-3} kJ/(mol·K))

T = absolute temperature (K)

M_{O2} = initial oxygen charge (kg initial oxygen/kg initial dry biomass)

β_{ij} = exponent (dimensionless)

The integrated form of Eq. 20 is

$$Y_i = \sum_j Y_{ij0} \exp(-k_{ij} M_{O2}{}^{\beta ij} t) \tag{22}$$

where

Y_{ij0} = yield of Component *ij* at time zero (kg residual component *ij*/kg initial Component *i*)

The Levenberg-Marquardt technique (LM), Simulated Annealing (SA), Interior Point methods (IP), the Greedy (G) Algorithm and several combinations of these methods were used to calculate parameters simultaneously (i.e., stepwise calculation of parameters was not applied). The objective function was the square sum of residuals, calculated as $R = \sum (y - \hat{y})^2$ where *y* is the observed data and $\hat{y}$ is the model estimate. The parameter search was extensive and systematic because several local minima were found for different initial guesses. IP gave the best results; thus, the parameters obtained using these method are reported here. Because parameter search was extensive, there is a good chance that the reported parameters are near the global minimum of the objective function. Details on parameter search methods and results are reported elsewhere.[156]

Additionally, the two models were compared on the basis of the highest F_c as suggested by Froment and Bischof:[157]

$$F_c = \frac{\sum_{i=1}^{n} \frac{\hat{y}_i^{\,2}}{p}}{\sum_{i-1}^{n} \frac{(y_i - \hat{y}_i)^2}{n-p}} \tag{23}$$

where

$\hat{y}_i$ = estimated value of dependent value

p = number of parameters in the model

n = number of experiments

y_i = measured data

Results and discussion

Lignin, glucan, and xylan degradations are direct functions of pretreatment time, temperature, and initial oxygen pressure.[111] Parameters for both models with their corresponding confidence intervals are shown in Tables 22, 23, and 24 (lignin, glucan, and xylan, respectively). Confidence intervals are wide especially for most frequency factors; however, small confidence interval half-widths for the predicted variable were observed (footnotes in Tables 22, 23 and 24). Confidence intervals for the predicted variable and for parameters were slightly smaller for Model 1, except for xylan. Temperature dependence followed the Arrhenius equation with regression coefficients of 1.0 because all parameters were found simultaneously.

Data fit for Model 1 is shown in Figures 39, 41, and 42 for lignin, glucan, and xylan respectively. Model 2 fit of data was very similar.[121] Model 1 has slightly lower R than Model 2 except for xylan; however, for all lignin, glucan, and xylan, Model 1 has significantly lower F_c than Model 2 (Table 21). This is because F_c favors a model for parsimony (i.e., simpler with fewer of parameters). According to the F_c criteria, Model 1 is preferred for all components of interest: lignin, glucan, and xylan.

Table 21. F_c and sum of residuals for Models 1 and 2

	F_c		Sum of residuals	
Model	Two moieties (Model 1)	Three moieties (Model 2)	Two moieties (Model 1)	Three moieties (Model 2)
Lignin	1060	653	0.147	0.146
Glucan	5005	3330	0.578	0.527
Xylan	6670	4830	0.344	0.289

Lignin degradation. At 140°C and pretreatment times >200 min, the model overestimates delignification. At 160°C and the same time interval, the model underestimates delignification. A fair representation of the data is observed. At 180°C, all pretreatment times and pressures, except for 600 min where delignification is overestimated (Figure 39).

Average Y_{Lf0} = 0.586 g lignin/g lignin in raw biomass (Table 22); however, variations were observed depending on temperature and pressure. As shown in Figure 39, at ~250 min and T ≤160°C, high slope (fast moiety) changes to low slope (slow moiety). Interestingly, at 180°C, two clear changes of slope become apparent, one at ~100 min and the other at ~400 min (Figure 40). These two changes are well estimated by Model 1.

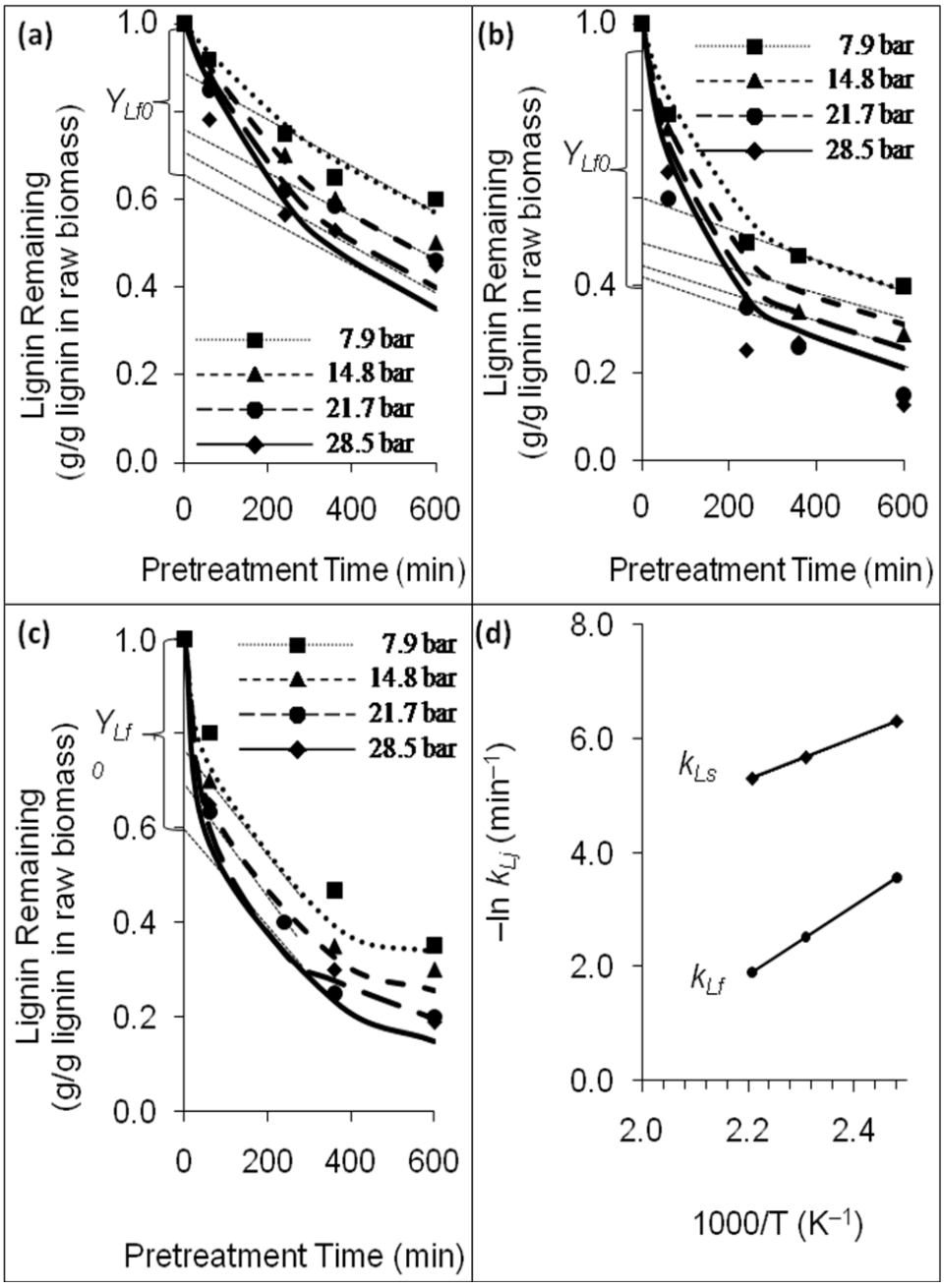

Figure 39. Data fit for lignin degradation using Model 1 (a) 110°C (b) 140°C (c) 160°C and (d) 180°C.

Table 22. Parameter estimates and confidence intervals for lignin using Models 1 and 2 ($\alpha = 0.05$)

Parameter	Units	Parameters ± confidence intervals	
		Model 1[a]	Model 2[b]
Y_{Lf0}	$\frac{\text{g lignin remaining}}{\text{g lignin in raw biomass}}$	0.586 ± 0.17	0.430 ± NA [d]
a_{Lf}	min^{-1}	5683 ± 28813	1022 ± 24829
E_{Lf}	kJ/mol	45.7 ± 11.5	39.5 ± 95.5
β_{Lf}	dimensionless	0.514 ± 0.268	0.382 ± 5.76
Y_{Lm0}	$\frac{\text{g lignin remaining}}{\text{g lignin in raw biomass}}$	–	0.395 ± 0.451
a_{Lm}	min^{-1}	–	2707 ± 13403
E_{Lm}	kJ/mol	–	44.6 ± 18.4
β_{Lm}	dimensionless	–	1.16 ± 1.12
Y_{Ls0}	$\frac{\text{g lignin remaining}}{\text{g lignin in raw biomass}}$	0.414 ± NA [c]	0.175 ± 0.442
a_{Ls}	min^{-1}	87.5 ± 1096	138 ± 3092
E_{Ls}	kJ/mol	35.6 ± 39.9	38.0 ± 82.0
β_{Ls}	dimensionless	1.41 ± 1.27	1.57 ± 3.40

[a] 95% confidence interval half-widths for the predicted variable (Y_L) varied between 0 and 0.0678
[b] 95% confidence interval half-widths for the predicted variable (Y_L) varied between 0 and 0.0852
[c] Calculated as $1-Y_{Lf0}$
[d] Calculated as $1- Y_{Lm0}- Y_{Ls0}$

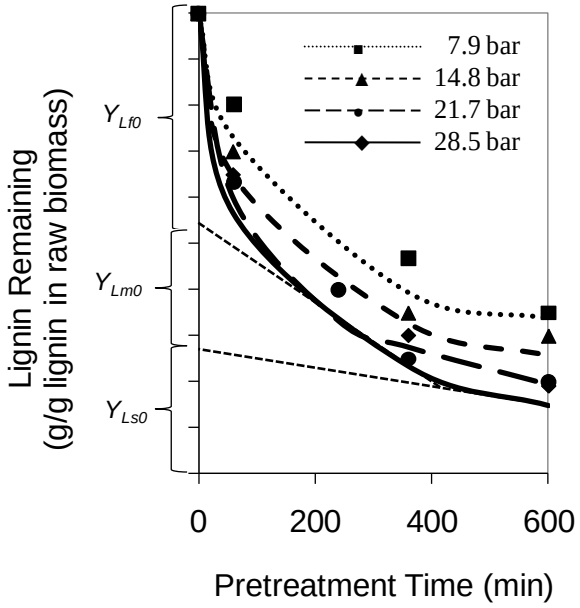

Figure 40. Two slope changes for lignin degradation at 180°C.

It is possible that at $T \leq 160$°C and $t > 600$ min, lignin degradation involves two changes of slope as well; however, this time range is out of the range of this study. The curves may level off because of condensation reactions, which promote C-C bonds triggered by phenoxy radicals. As a result, the remaining lignin becomes almost inert.

At 180°C, 360 min, and 28.5 bar, fast-degrading lignin (Y_{Lf0} = 0.447 g lignin/g lignin in raw biomass) degraded completely. At the most severe pretreatment conditions in this study (i.e., 180°C, 28.5 bar, and 600 min), 34% of the initial amount of slow-degrading lignin (Y_{Ls0} = 0.553 g lignin/g lignin in raw biomass) still remained unreacted

The rate constant for fast-degrading lignin (k_{Lf}) was 15 to 30 times greater than the corresponding k_{Ls} with the largest differences observed at the highest temperatures. Activation energies were 46 and 36 kJ/mol for fast-degrading and slow-degrading

moieties, respectively, which is similar to values reported in the literature.[162] However, these values are low compared to values normally observed in most chemical reactions indicating that diffusion processes may control rather than chemical reactions.

The reaction order for slow-degrading oxygen β_{Ls} was about 3 times greater than β_{Lf} indicating that oxygen is needed when degrading less-reactive (slow) lignin moieties. Phenolic moieties in lignin – highly recalcitrant and unreactive otherwise– are effectively degraded by oxygen in alkaline media; thus, oxygen is more required during slow- degradation stages. Furthermore, analogous work with the aim of modeling delignification in oxidative alkaline media have also reported $\beta_{Ls} > \beta_{Lf}$.[147] Because of the need for oxygen, lignin degradation may be importantly improved by enhanced mixing.

Glucan degradation. At 140°C, initial oxygen pressure ≥21.7 bar, and for all pretreatment times, the model tends to underestimate glucan degradation. At 160°C, initial oxygen pressure ≤14.8 bar, and for all pretreatment times, the model tends to overestimate glucan degradation. At 180°C, all pretreatment times and pressures, a fair representation of the data is observed (Figure 41).

As shown in Figure 41, average Y_{Lf0} = 0.123 g glucan/g glucan in raw biomass (Table 22) varied proportional to temperature and pressure. Also, the change from high slope (fast- degrading glucan) to low slope (slow-degrading glucan) occurred at 50 min independently of temperature. This is much earlier than the change of slope observed for lignin and xylan.

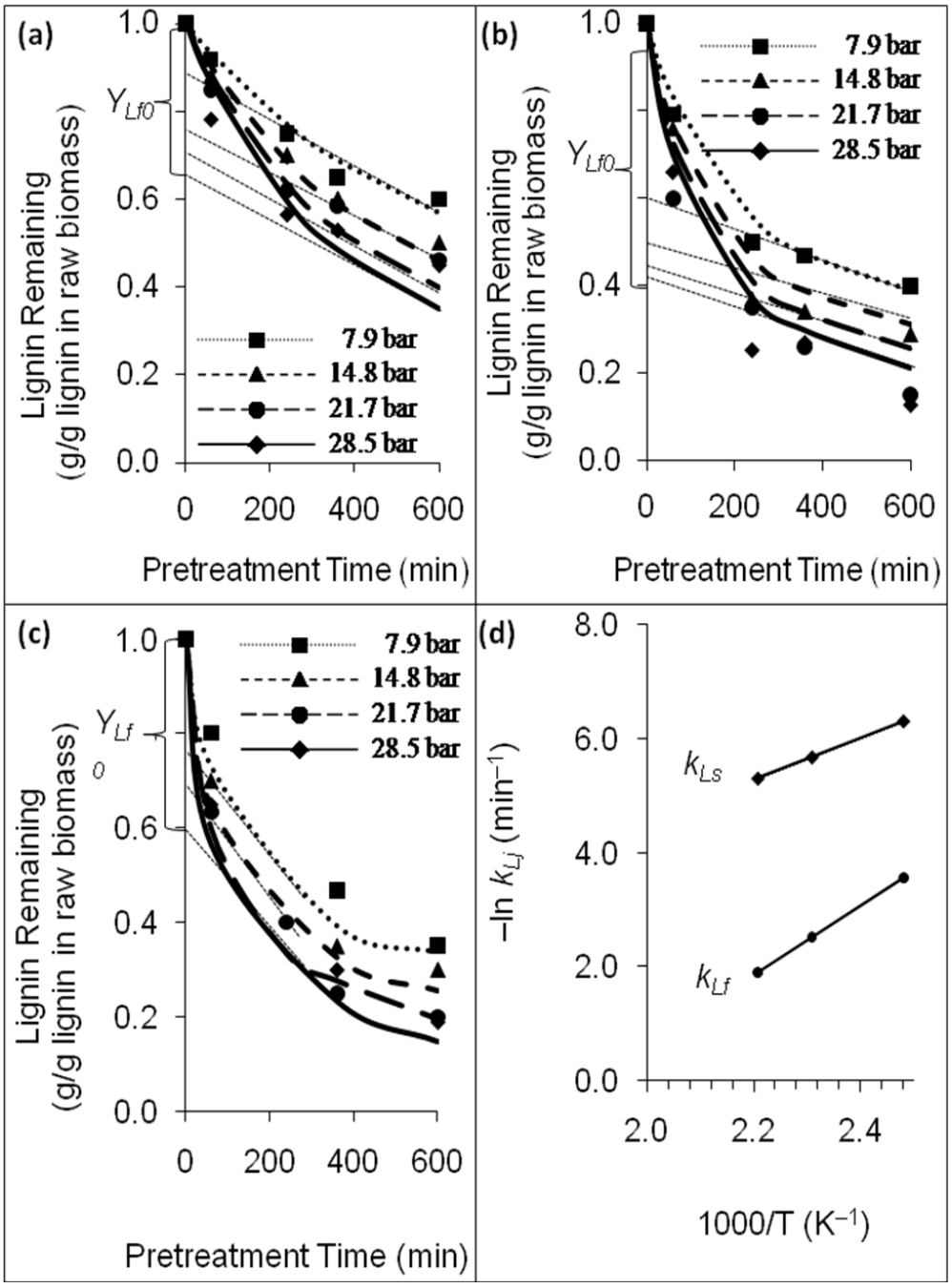

Figure 41. Data fit for glucan degradation using Model 1 (a) 110°C (b) 140°C (c) 160°C and (d) 180°C.

Table 23. Parameter estimates and confidence intervals for glucan using Models 1 and 2 ($\alpha = 0.05$)

Parameter	Units	Parameters ± confidence intervals	
		Model 1[a]	Model 2[b]
Y_{Gf0}	$\frac{\text{g glucan remaining}}{\text{g glucan in raw biomass}}$	0.123 ± NA [c]	0.0710 ± NA [d]
a_{Gf}	min^{-1}	$4.23\times10^{7} \pm 2.99\times10^{8}$	1928 ± 39882
E_{Gf}	kJ/mol	54.9 ± 34.9	32.5 ± 74.7
β_{Gf}	dimensionless	5.54 ± 2.34	2.5 ± 5.10
Y_{Gm0}	$\frac{\text{g glucan remaining}}{\text{g glucan in raw biomass}}$	–	0.0540 ± 0.150
a_{Gm}	min^{-1}	–	$2.42\times10^{11} \pm 9.27\times10^{13}$
E_{Gm}	kJ/mol	–	45.4 ± 149
β_{Gm}	dimensionless	–	14.8 ± 301
Y_{Gs0}	$\frac{\text{g glucan remaining}}{\text{g glucan in raw biomass}}$	0.877 ± 0.0395	0.874 ± 0.0500
A_{Gs}	min^{-1}	$1.91\times10^{11} \pm 1.18\times10^{13}$	$5.36\times10^{11} \pm 1.02\times10^{13}$
E_{Gs}	kJ/mol	122 ± 58.6	126 ± 71.4
β_{Gs}	dimensionless	0.663 ± 0.350	0.751 ± 0.550

[a] 95% confidence interval half-widths for the predicted variable (Y_G) varied between 0 and 0.103
[b] 95% confidence interval half-widths for the predicted variable (Y_G) varied between 0 and 0.153
[c] Calculated as $1-Y_{Gs0}$
[d] Calculated as $1- Y_{Gm0}- Y_{Gs0}$

This result is not surprising because most cellulose loss is expected at the beginning of pretreatment because of peeling reactions. Subsequent stopping reactions prevent sugars from further degradation.[95] Accordingly, at $T \leq 160$°C and $t > 50$ min, glucan degradation levels off, becoming almost independent of time.

Oppositely, at $T = 180$°C and $t > 50$ min the glucan degradation rate is much higher. As shown here and in previous studies, the effect of temperature is significant on cellulose degradation.[135, 147]

At 140°C, 28.5 bar, and 240 min, the fast-degrading glucan fraction Y_{Gf0} completely degraded, whereas the slow-degrading glucan fraction was more recalcitrant to degradation. At 180°C, 28.5 bar, and 600 min (the most severe conditions in this study), 60% of Y_{Gs0} still remained unreacted.The rate constant for fast-degrading glucan k_{Gf} was 12,300 to 1.12×10^5 times higher than the corresponding k_{Gs} with the highest differences observed at the lowest temperatures. Activation energies were 55 and 122 kJ/mol for fast-degrading and slow-degrading glucan, respectively. These values (particularly the second) are expected for most chemical reactions indicating that glucan degradation is chemically controlled rather than mass transfer controlled.

The oxygen reaction order for fast-degrading glucan (β_{Gf}) is ~8 times greater than β_{Gs} indicating that initial fast degradation of glucan is importantly enhanced by oxygen. Afterwards, oxygen does not affect glucan degradation as much, which is opposite to lignin degradation.

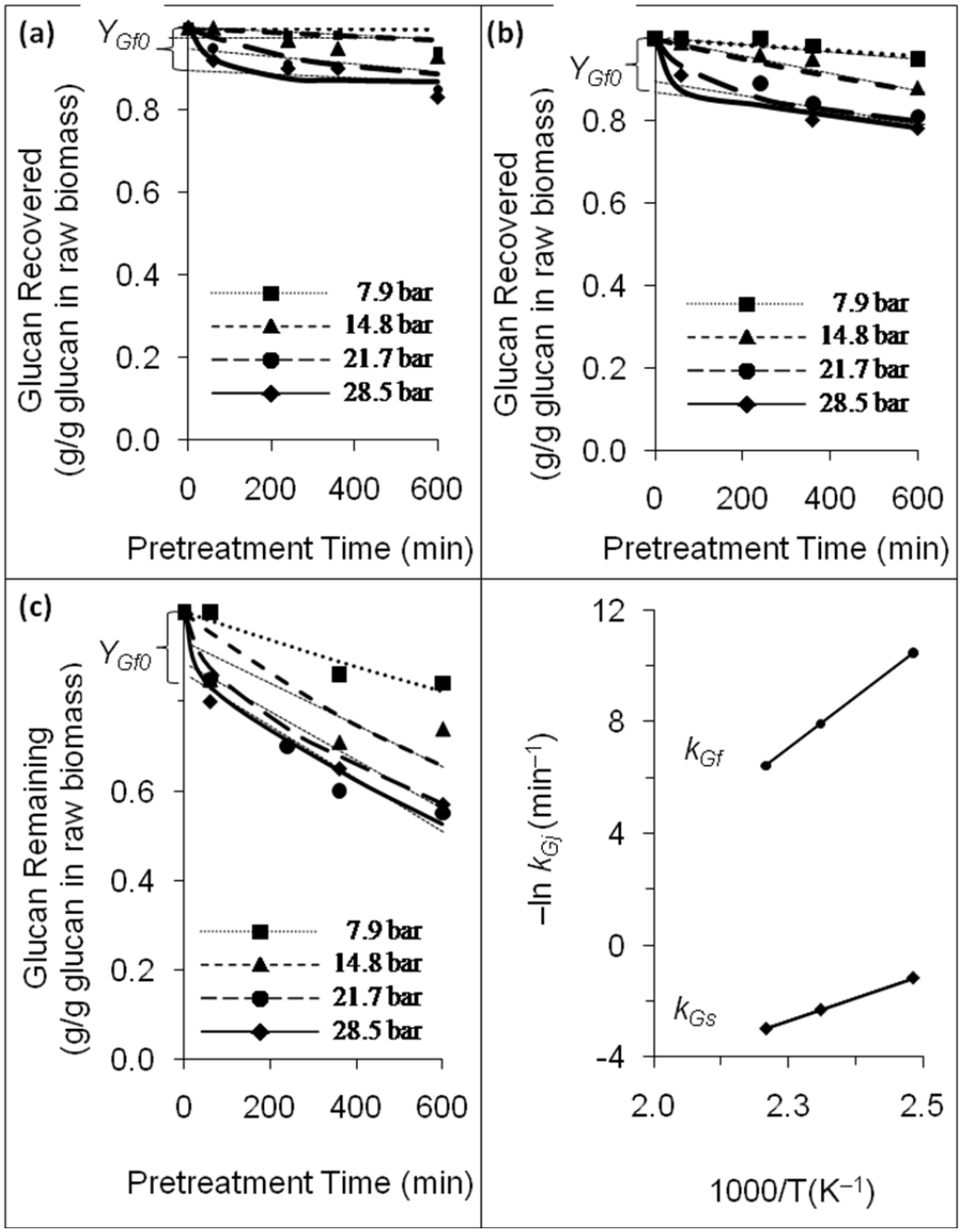

Figure 42. Data fit for xylan degradation using Model 1 (a) 110°C (b) 140°C (c) 160°C and (d) 180°C.

Table 24. Parameter estimates and confidence intervals for xylan using Models 1 and 2 ($\alpha = 0.05$)

Parameter	Units	Parameters ± confidence intervals	
		Model 1[a]	Model 2[b]
Y_{Xf0}	$\frac{\text{g xylan remaining}}{\text{g xylan in raw biomass}}$	0.447 ± 0.423	0.110 ± 0.0893
a_{Xf}	min^{-1}	4.24×10^{4} ± 1.04×10^{5}	0.0389 ± 0.588
E_{Xf}	kJ/mol	56.7 ± 9.29	1.91 ± 52.1
β_{Xf}	dimensionless	0.521 ± 0.197	1.06 ± 1.02
Y_{Xm0}	$\frac{\text{g xylan remaining}}{\text{g xylan in raw biomass}}$	–	0.622 ± 0.119
a_{Xm}	min^{-1}	–	1.48×10^{8} ± 8.01×10^{8}
E_{Xm}	kJ/mol	–	89.8 ± 19.7
β_{Xm}	dimensionless	–	0.299 ± 0.271
Y_{Xs0}	$\frac{\text{g xylan remaining}}{\text{g xylan in raw biomass}}$	0.553 ± NA [c]	0.268 ± NA [d]
a_{Xs}	min^{-1}	1.76×10^{9} ± 3.04×10^{12}	88.1 ± 2522
E_{Xs}	kJ/mol	104 ± 144	24.3 ± 76.7
β_{Xs}	dimensionless	0.419 ± 0.370	6.07 ± 17.0

[a] 95% confidence interval half-widths for the predicted variable (Y_X) varied between 0 and 0.135
[b] 95% confidence interval half-widths for the predicted variable (Y_X) varied between 0 and 0.0394
[c] This parameter was calculated as $1-Y_{Xf0}$
[d] This parameter was calculated as $1- Y_{Xf0}- Y_{Xm0}$

Using this result and the previously discussed results for E_{Gf} and E_{Gs}, it is inferred that most glucan degradation is controlled by chemical reactions in which oxygen is barely involved and consequently possible mass transfer issues are minor.

Xylan degradation. Parameters for Models 1 and 2 are shown in Table 24. Model 1 represents xylan degradation data very well at all temperatures, pressures, and times, but a small overestimation was observed at 160°C, $P \geq 21.7$ bar, and, $t \geq 400$ min (Figure 42). At $t \sim 250$ min and $T \leq 160$°C, high slope (fast moiety) changes to low slope (slow moiety).

At the same temperatures, these times coincide with the change of slope for lignin degradation. Furthermore, at 180°C, two clear changes of slope become apparent, one at ~50 min and the other at ~400 min (Figure 43). The first change of slope coincides with the change of slope observed for glucan degradation (compare Figures 41 and 43), whereas the second change of slope coincides with the second change of slope in lignin degradation at the same temperature (compare Figure 43 to Figure 40).

This evidence shows that at the beginning of pretreatment, xylan degradation is concurrent and may be related glucan degradation. This stage is characterized by rapid peeling attack on carbohydrates. Conversely, for harder-to-degrade xylan moieties (longer pretreatments), chemical bonds between hemicellulose and lignin promote simultaneous lignin and xylan removal, whereas glucan is more stable.

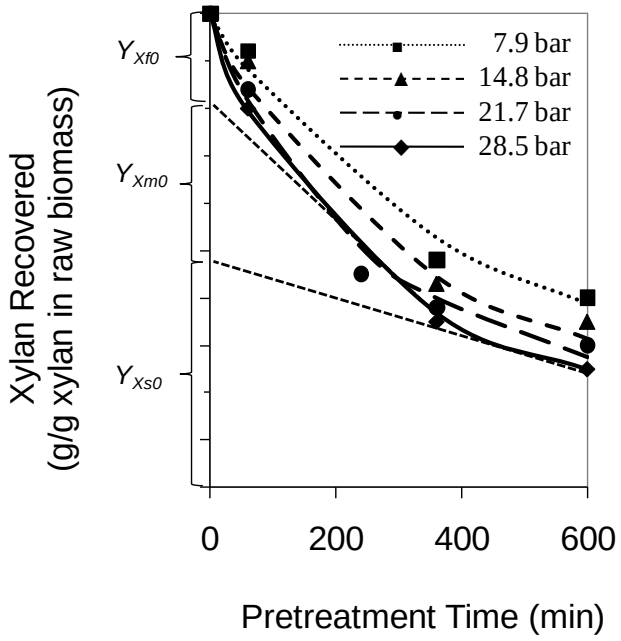

Figure 43. Two slope changes for xylan degradation at 180°C.

At 180°C, P ≥14.8 bar, and 600 min, the fraction of fast-degrading xylan (Y_{Lf0} = 0.447 g xylan/g xylan in raw biomass) was completely degraded. At 180°C, 28.5 bar, and 600 min, the slow-degrading xylan was maximally removed with ~45% of this xylan fraction remaining unreacted.

For fast-degrading xylan, the rate constant k_{Xf} was 10 to 30 times higher than the corresponding k_{Xs} with the highest differences observed at the lowest temperatures. Activation energies were 57 and 105 kJ/mol, which is very close to E_{ij} observed of glucan degradation; thus, as opposed to lignin degradation, carbohydrate degradation was chemically rather than mass transfer controlled.

Unlike lignin and glucan degradation, the reaction order for fast (β_{Xf}) and slow (β_{Xs}) xylan degradation were low and comparable among them (i.e., β_{Xf} = 0.52 and β_{Xs} = 0.41). This indicates that xylan degradation is not as affected by oxygen as lignin and glucan degradation, a result that has been also observed in other modes of lime pretreatment that are scarce in oxygen.[163] This also agrees with the previous statement that xylan degradation reactions are controlled chemically rather by than mass transfer.

Model assessment

Mass profiles compare the selected Model 1 to the experimental data for lignin, glucan, and xylan (Figure 44). Also, most data fit the model within 10% (Figure 45) and all statistics (summarized in Table 20 for R and F_c and in Tables 21, 22, for parameters with confidence intervals for lignin, glucan, and xylan, respectively) give good evaluation of the selected model, except for wide confidence intervals for some parameters.

Selectivity

Glucan and xylan selectivity measure the ability of pretreatment to remove lignin while retaining carbohydrates. In this study, glucan and xylan selectivities were calculated using Model 1 in two forms: differential (ratio of lignin degradation rate to carbohydrate degradation rate) and integral (ratio of lignin removed to carbohydrate removed).

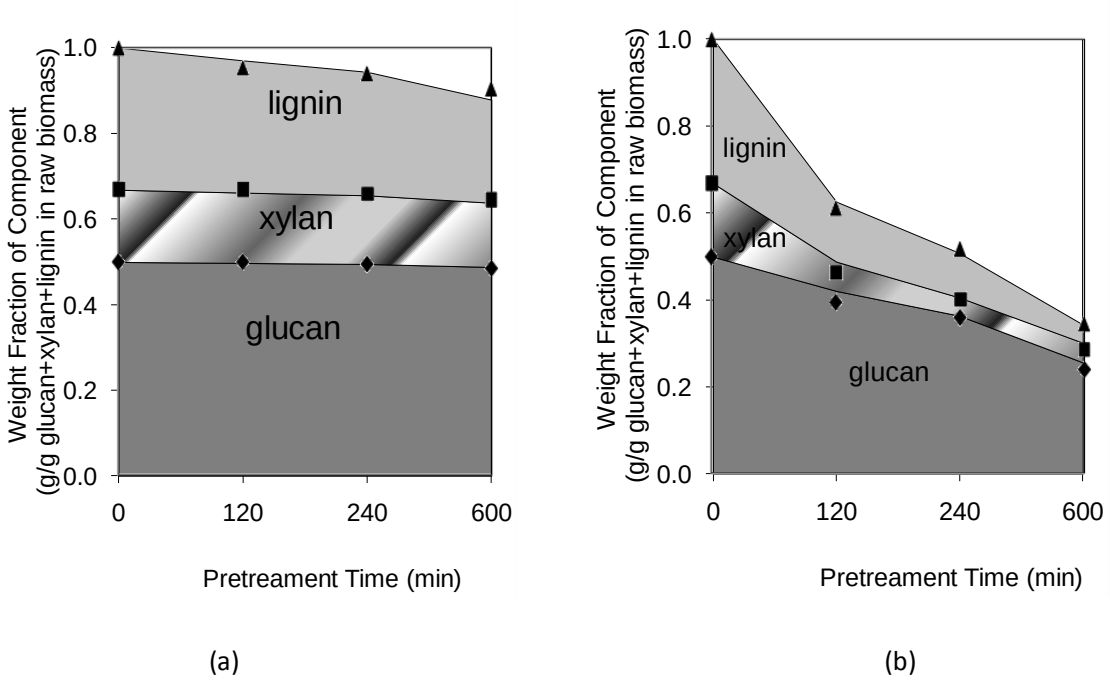

Figure 44. Experimental (data points) and model estimated (continuous lines) mass profiles for glucan, lignin, and xylan at (a) 140°C and 7.9 bar (b) 180°C and 28.5 bar.

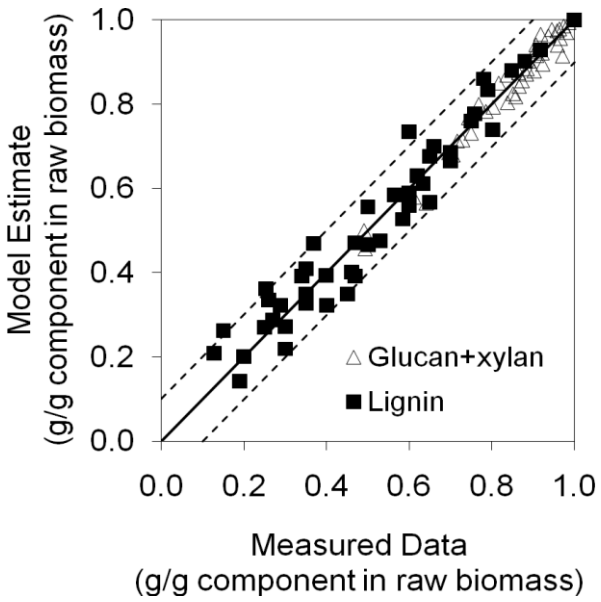

Figure 45. Model assessment. Dotted lines describe 95% prediction intervals.

Differential selectivity is calculated as follows:

$$S_{dG} \equiv \frac{dY_L/dt}{dY_G/dt} = \frac{a_{Lf}\exp\left(-\frac{E_{Lf}}{RT}\right)M_{O2}^{\beta Lf}Y_{Lf} + a_{Ls}\exp\left(-\frac{E_{Ls}}{RT}\right)M_{O2}^{\beta Ls}Y_{Ls}}{a_{Gf}\exp\left(-\frac{E_{Gf}}{RT}\right)M_{O2}{}^{\beta Gf}Y_{Gf} + a_{Gs}\exp\left(-\frac{E_{Gs}}{RT}\right)M_{O2}{}^{\beta Gs}Y_{Gs}}$$

(24)

$$S_{dX} \equiv \frac{dY_L/dt}{dY_X/dt} = \frac{a_{Lf}\exp\left(-\frac{E_{Lf}}{RT}\right)M_{O2}^{\beta Lf}Y_{Lf} + a_{Ls}\exp\left(-\frac{E_{Ls}}{RT}\right)M_{O2}^{\beta Ls}Y_{Ls}}{a_{Xf}\exp\left(-\frac{E_{Xf}}{RT}\right)M_{O2}^{\beta Xf}Y_{Xf} + a_{Xs}\exp\left(-\frac{E_{Xs}}{RT}\right)M_{O2}^{\beta Xs}Y_{Xs}} \quad (25)$$

The highest differential glucan selectivities are observed at the beginning of pretreatment, when the lignin content is high, at low temperatures, and low pressures (Figure 46). At 140°C, selectivities are significantly higher than those observed at corresponding pressures and pretreatment times for 160°C, which in turn is somewhat higher than corresponding selectivities at 180°C. Selectivity decreases with pressure; however, the differences in selectivity among different pressures are not as important as in the case of temperature.

The highest differential selectivity was 221 g lignin degraded/g glucan degraded and was observed at 140°C, 7.9 bar, and at the beginning of pretreatment. The lowest selectivity was ~0.5 g lignin degraded/g glucan degraded and similar values were observed at 180°C for all pressures and 600 min. A treatment temperature of 180°C is not advised for the following reasons: (1) the highest observed selectivity is ~12 times

smaller than the highest selectivity at 140°C, and (2) selectivity ≤1 g lignin degraded/g glucan degraded for all pressures, at pretreatment times ≥360 min.

Similar to differential glucan selectivity, the highest differential xylan selectivities were observed at low temperature and pressure, and at the beginning of pretreatment when lignin content is high (Figure 46).

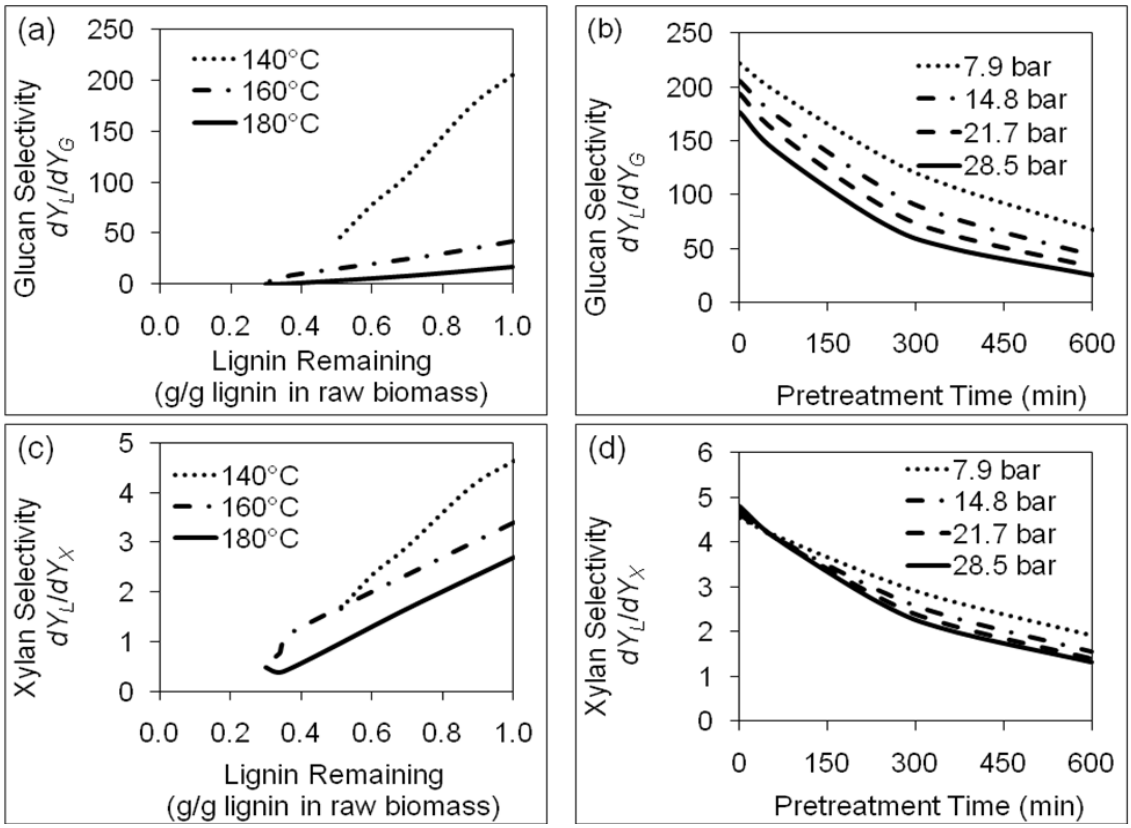

Figure 46. Differential selectivity (a) for glucan at 14.8 bar total pressure (b) for glucan at 140°C (c) for xylan at 14.8 bar total pressure (d) for xylan at 140°C.

The highest and lowest were 5.2 and 1.2 g lignin removed/g xylan removed. These extreme values were observed at pretreatment conditions corresponding to those

where the highest and lowest glucan selectivities occurred. In some instances, glucan selectivity was up to ~50 times higher than xylan selectivity, but in other cases – particularly for high temperature, pressure and pretreatment time– they are about the same (Figure 46).Xylan selectivity is not as sensitive to pretreatment conditions as glucan selectivity, i.e., the differences in selectivity observed for different temperatures, pressures, and pretreatment times are not as remarkable as those observed for glucan selectivity.

Integral selectivity is calculated as follows:

$$S_G \equiv \frac{1-Y_L}{1-Y_G} = \frac{1-Y_{Lf0}\exp\left(-a_{Lf}\exp\left(-\frac{E_{Lf}}{RT}\right)M_{O2}^{\beta Lf}t\right)-Y_{Ls0}\exp\left(-a_{Ls}\exp\left(-\frac{E_{Ls}}{RT}\right)M_{O2}^{\beta Ls}t\right)}{1-Y_{Gf0}\exp\left(-a_{Gf}\exp\left(-\frac{E_{Gf}}{RT}\right)M_{O2}^{\beta Gf}t\right)-Y_{Gs0}\exp\left(-a_{Gs}\exp\left(-\frac{E_{Gs}}{RT}\right)M_{O2}^{\beta Gs}t\right)}$$

(26)

$$S_X \equiv \frac{1-Y_L}{1-Y_X} = \frac{1-Y_{Lf0}\exp\left(-a_{Lf}\exp\left(-\frac{E_{Lf}}{RT}\right)M_{O2}^{\beta Lf}t\right)-Y_{Ls0}\exp\left(-a_{Ls}\exp\left(-\frac{E_{Ls}}{RT}\right)M_{O2}^{\beta Ls}t\right)}{1-Y_{Xf0}\exp\left(-a_{Xf}\exp\left(-\frac{E_{Xf}}{RT}\right)M_{O2}^{\beta Xf}t\right)-Y_{Xs0}\exp\left(-a_{Xs}\exp\left(-\frac{E_{Xs}}{RT}\right)M_{O2}^{\beta Xs}t\right)} \quad (27)$$

Integral selectivity behaves very similar to differential electivity, i.e., it decreases with pretreatment time, temperature, and pressure and the effect of temperature is greater than the effect of pressure; however, glucan integral selectivity can be as much as ~6 times higher than differential selectivity particularly at higher temperatures, pressures, and longer pretreatments (i.e., pretreatment at 160°C, 21.7 bar, and 600 min). Likewise,

xylan integral selectivity can be as much as ~4 higher than xylan differential selectivity, for instance at 180°C, 7.9 bar, and 600 min.

Conclusions

Kinetic modeling of pretreatment is essential for reactor design, control, and optimization. Oxygen bleaching of pulp is described by models that use a standard power law rate equation and consider two or three moieties reacting in parallel. The models correlate reaction rate with the main process variables, such as alkali concentration, temperature, and oxygen pressure. In this study, lime was always used in excess. Because lime is sparingly soluble in water, the alkali concentration [OH^-] is constant and therefore is not included as a variable. Through this study, parameters were found that fit these models to experimental data obtained for oxidative lime pretreatment of poplar wood using a variety of temperature, times, and oxygen loadings. Based on best fit and statistical analysis, Model 1 with two moieties (fast and slow) was preferred over Model 2 with three moieties (fast, medium, and slow) for lignin, glucan, and xylan.

For both models, activation energies for delignification were between 35 and 45 kJ/mol. This is slightly less than previous kinetic studies on oxidative-lime pretreatment, which reported activation energies close to 50 kJ/mol.[151] Also, considering both models, activation energies for glucan degradation varied from 32 to 122 kJ/mol and for xylan between 24 and 124 kJ/mol, excluding E_{Xf} = 1.91 kJ/mol obtained for Model 2.

Even though both models fit data adequately, Model 1 was selected on the basis of highest F_c and was used to calculate glucan and xylan selectivity in two fashions: differential and integral. In general, higher selectivities were observed at low temperature, high lignin content, low pressures, and at the beginning of pretreatment.

In the case of differential selectivity, glucan was ~50 times higher than xylan. Integral selectivities showed the same tendencies and were similar to differential selectivities, but in some instances were up to ~6 times higher.

SELECTIVITY AND DELIGNIFICATION KINETICS FOR OXIDATIVE AND NON- OXIDATIVE LIME PRETREATMENT OF POPLAR WOOD. PART III: LONG-TERM*

Synopsis

Lime pretreatment is an effective method for improving lignocellulose digestibility by removing lignin. For several weeks, mixtures of poplar wood, water, and calcium hydroxide (lime) were submitted to temperatures from 25 and 65°C, with and without aeration. Kinetic models for lignin and carbohydrate degradation were obtained as functions of temperature, time, and aeration using first-order kinetics in lignin and carbohydrates. Model 1 considered two reacting moieties (slow and fast) and Model 2 considered three (slow, medium and fast).

Model 1 was statistically better and was employed to determine differential and integral selectivities, which measure the ability of pretreatment to retain carbohydrates while degrading lignin. During the first two weeks, when lignin content ≥0.80 g/g lignin in raw biomass, both glucan and xylan differential and integral selectivities decreased rapidly. Afterwards, selectivities were nearly constant ranging between 0 and 3 g lignin removed/g carbohydrate degraded.

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Introduction

Lignocellulosic biomass is the most widely available source for carbohydrates, a fermentation substrate for the production of fuels and chemicals; however, this feedstock is not readily digestible. To overcome this difficulty, lignocellulose structure must be modified through pretreatment.

In lime pretreatment, lignocellulosic biomass is mixed with calcium hydroxide and water, and exposed to temperatures ranging from 25 to 180°C, for hours to weeks, with or without an oxidizing agent (air or pressurized oxygen). This procedure modifies the biomass composition mainly by degrading lignin via reactions that are enhanced by an oxidative agent.[75, 161] If 1-atm air is used at pretreatment temperatures up to 65°C, oxygen solubility in water is low; nevertheless, important delignification enhancements have been reported.[11, 75, 90] Because of delignification, biomass swells leading to increased internal surface area and median pore volume, thereby improving digestibility.[16, 75, 111, 163]

A kinetic model is a key factor to design, optimize, and control the pretreatment process. An extensive background on kinetic models for kraft and bleaching is available.[22, 30, 105, 164] Recently, delignification kinetic models have been obtained for corn stover lime treated at temperatures between 25 and 55°C, atmospheric pressure, with/without aeration, for several weeks (i.e., long-term lime pretreatment).[151] On the basis of this background, this work develops delignification models for poplar wood pretreated at a maximum temperature of 65°C, with and without atmospheric pressure aeration, for several weeks. Wood has higher lignin content than many other materials;

thus, it is more recalcitrant and had not been evaluated in the mild conditions of long-term lime pretreatment.

Non-oxidative alkaline depolymerization of lignin mostly depends on the cleavage of two types of aryl ether bonds: C_{aliph}–O–C_{arom} and C_{arom}–O–C_{arom} (ordered from least to most stable), which frequently correspond to α- and β-aryl ether bonds (50–70% in wood). In addition to these, carbon-to-carbon bonds are also found, especially C_{arom}–C_{arom}, but these are very stable in a non-oxidative media.[22]

On the other hand, oxidative alkaline delignification involves the release of electrons, which is triggered in alkaline media at high temperatures. Oxygen delignification follows complex mechanisms including two major delignification pathways: (1) a dominant phenolic delignification and (2) a "peeling" delignification, which occurs at the reducing ends where hemicellulose is covalently bonded to lignin. Unlike the non-oxidative alkaline process, oxygen delignification attacks C–C bonds.[100]

Lime pretreatment at 25 to 65°C with aeration is expected to follow a combined delignification mechanism, involving both oxidative and non-oxidative depolymerization of lignin. The oxidative reactions are slow because of the low solubility of oxygen in water at the pretreatment conditions (1-atm air).

Non-oxidative cellulose and hemicellulose degradation start with an end-wise degradation (peeling), which is the dissolution of short-chain material detached from the reducing ends of molecules. This type of reaction is triggered in the presence of oxygen and proceeds slowly in cellulose and rapidly in hemicellulose. A competing reaction promotes stabilization of the cellulose (a 'stopping' reaction) through the establishment

of up to 16 different stabilizing acid terminal units,[165] whereas the peeling reaction of birch xylan proceeds until a xylose moiety carrying a 4-O-methylglucoronic acid group at C-2 is reached.[166]

Random alkaline scission (hydrolysis) of glycosidic linkages may also occur, especially in the presence of oxygen, but this requires temperatures that are much higher than the ones used in this study.[167]

Compared to other pretreatment options, long-term lime preatreatment uses much lower temperature and pressure, but much longer times.[16, 71] Because the reactor condition are mild, pretreatment can occur in simple reactors while biomass is being stored. Combined long-term pretreatment and fermentations in a set of low-cost fixed bed reactors has demonstrated high conversions.[82] More rapid modes of oxidative lime pretreatment use high-pressure oxygen to continually replenish liquid-phase oxygen as it reacts.[111]

This article is part of a four-paper series that describes kinetic models for oxidative lime pretreatment of poplar wood with the following topics: (I) constant-pressure pretreatment, which uses temperatures up to 180°C and constant oxygen pressures up to 21.7 bar for pretreatments lasting 1 to 6 hours;[162] (II) varying-pressure pretreatment, which uses temperatures up to 180°C and initial oxygen pressures up to 28.5 bar for pretreatments lasting 1 to 10 hours;[140] (III) low-temperature and atmospheric pressure, lasting several weeks (this study); and (IV) comparison and combination of different modes of lime pretreatment to recommend conditions that optimize selectivity.[112]

The aim of the present work is to derive kinetic .s for lignin and carbohydrate degradation during long-term lime pretreatment. The equations are used to calculate pretreatment selectivity.

Methods

Fixed-bed reactors were built using 2-inch-ID PVC pipes (0.219 L total capacity). The pretreatment temperature was maintained by circulating preheated water through a PVC jacket in the reactors. Five temperatures were tested: 25, 35, 45, 55 and 65°C. Once the reactors were at the desired temperature, a homogeneous mixture of water (150 mL), biomass (15 g dry basis), and $Ca(OH)_2$ (7.5 g) was placed in each reactor. In some cases, compressed air flowed through the reactors from the bottom at about 3.5 mL/min. In other cases, no air was bubbled and the reactors were closed during pretreatment (1 to 12 weeks). Pretreated and untreated poplar woods were submitted to compositional analysis following the National Renewable Energy Laboratory Analytical Procedures. A thorough discussion on equipment set up, experimental design, pretreatment method, analytical laboratory procedures, comparisons and relationships between the observed lignin, glucan, and xylan degradations for both aerated and non-aerated modes of long-term lime pretreatment, including statistical analysis can be found elsewhere.[163]

The pretreatment yields of lignin, glucan, and xylan are defined as follows:

$$Y_i \equiv \frac{C_i \cdot Y_T}{C_{i_0}} \tag{28}$$

where

i = lignin L, glucan G, or xylan X

Y_i = pretreatment yield of Component i at time t (kg residual Component i/kg Component i in raw biomass)

C_{i0} = Component i content at time zero (kg Component i in raw biomass/kg raw biomass)

C_i = Component i content at time t (kg residual Component i/kg residual biomass)

Y_T = total solids pretreatment yield at time t (kg residual biomass/kg raw biomass)

Estimation of kinetic parameters

A kinetic model that describes the degradation of both lignin and carbohydrates is used in simulations that provide insights into the effects of long-term lime pretreatment to identify conditions that selectively remove lignin while preserving carbohydrates. Several kinetic models of varying complexity for lignin and carbohydrate degradation during oxygen bleaching have been developed.[135, 146, 149] Many use a standard power rate equation that considers the main process variables, such as alkali concentration, temperature, and oxygen pressure. Mass transfer resistances are neglected, or are implicitly included with the kinetic parameters, thus narrowing their applicability to conditions identical to those used to generate the experimental data.[143]

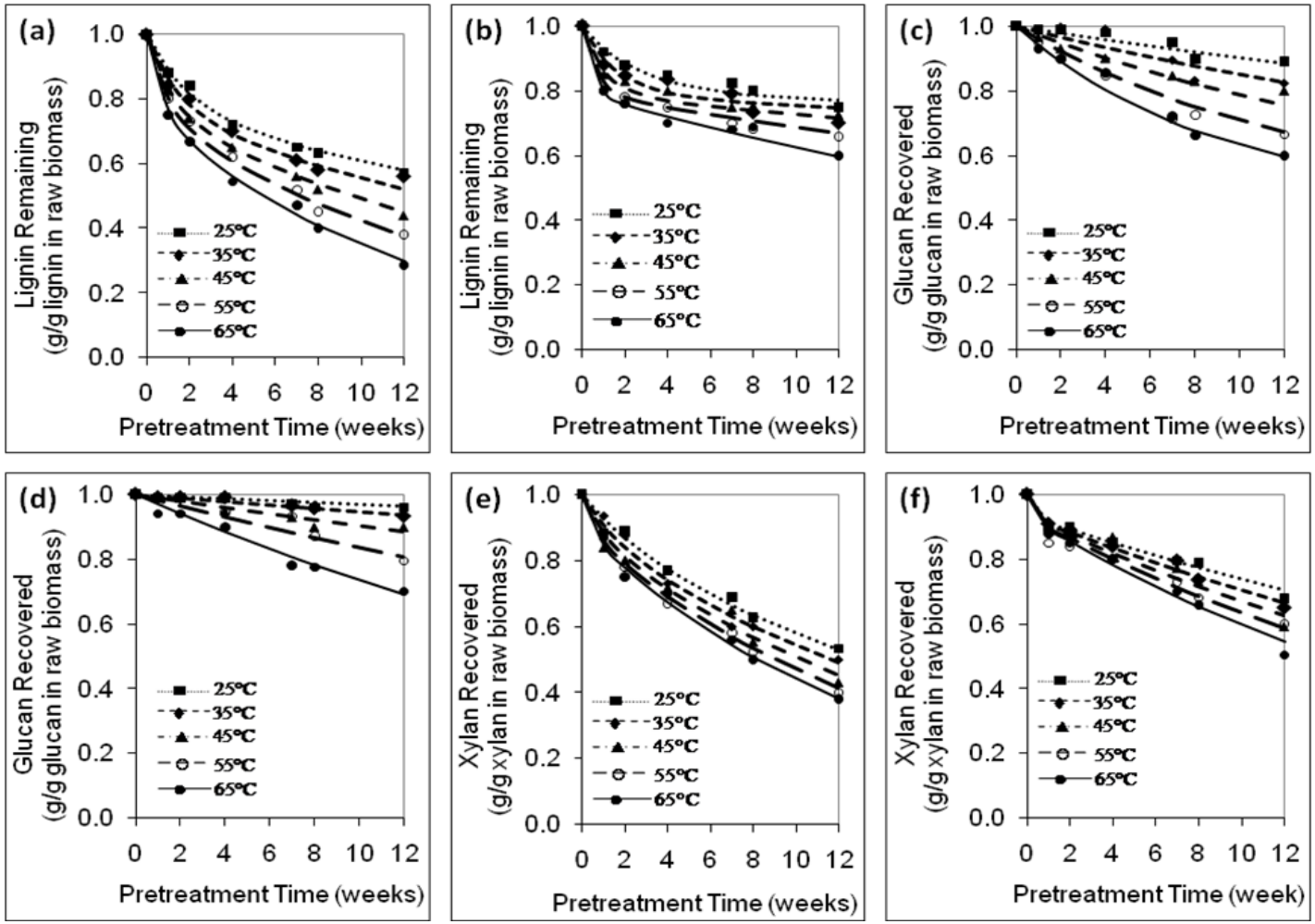

Figure 47. Data fit for degradation of (a) lignin oxidative (b) lignin non-oxidative (c) glucan oxidative (d) glucan non-oxidative (e) xylan oxidative (f) xylan non-oxidative.

As discussed in Parts I and II of this series, two such models were considered in this study. They are first order in the degrading component and include either two (*Model 1*) or three (*Model 2*) terms to describe moieties with different reactivity: fast (*f*), medium (*m*), and slow (*s*).

From a reaction mechanism viewpoint, varying reactivity is explained y the fact that lignin and carbohydrate degradation starts with the most readily available reacting moieties. Other moieties in the polymers are more inert. For example, C-C links in lignin are harder to attack than C-aromatic links; thus, reaction are much slower. Furthermore, after reaching a certain extent of degradation, condensation and termination reactions compete with further degradation reactions, which significantly reduces reaction rate.[22] These effects are readily seen when lignin and carbohydrate concentrations are plotted versus time (Figure 47). It is easy to identify a rapidly degrading (i.e., high-slope) zone followed by a slowly degrading (i.e., low-slope) zone.

For lime pretreatment, the model is based on the following definition of concentration of lignin and carbohydrates:

$$Y_i = \sum_j Y_{ij} \tag{29}$$

where

i = L for lignin, G for glucan, and X for xylan

j = f and s (Model 1) and f, m, and s (Model 2)

Y_{ij} = yield of Component i at time t (kg residual Component i/kg initial

Component *i*)

At time zero,

$$Y_{i0} = \sum_j Y_{ij0} = 1 \tag{30}$$

Component *i* degradation is modeled as:

$$-\frac{dY_i}{dt} = \sum_j k_{ij} Y_{ij} \tag{31}$$

where

$$k_{ij} = a_{ij} \exp\left(-\frac{E_{ij}}{RT}\right) \tag{32}$$

and

k_{ij} = rate constant (min^{-1})

a_{ij} = frequency factor (min^{-1})

E_{ij} = activation energy (kJ/mol)

R = ideal gas constant (8.314×10^{-3} kJ/(mol·K))

T = absolute temperature (K)

The integrated form of Eq. 31 is

$$Y_i = \sum_j Y_{ij0} \exp(-k_{ij} t) \tag{32}$$

where

Y_{ij0} = yield of Component i (lignin, glucan, or xylan) moiety j (fast, medium, or slow) at time zero (kg residual Component ij/kg initial Component i)

Although models for both oxidative and non-oxidative delignification use the same eq. 6, their parameters are significantly different because the presence of air changes the degradation mechanisms, and therefore the reaction rate, as explained in the introduction section.

In Parts I and II, Models 1 and 2 are different from Eq. 32 because they include a variable to account for oxygen pressure. In this Part III, this variable is not included. In oxidative pretreatment, the partial pressure of oxygen is fixed according to the temperature; consequently, its effect is included in the rate constant k_{ij}. In non-oxidative pretreatment, oxygen does not participate in the reaction. Additionally, because alkali was applied in great excess and is sparingly soluble in water, [OH^-] may be assumed as constant and is included in k_{ij}.

For these non-linear kinetic equations, Matlab R-12® (*lsqnonlin* command) was used to estimate parameters on the basis of the residual sum of squares (*R*) calculated as $R = \sum (y - \hat{y})^2$ where y is the observed data and $\hat{y}$ the model estimated. A deterministic method, the Levenberg-Marquardt technique (LM), was initially implemented and all parameters were estimated simultaneously (i.e., stepwise calculation of parameters was not applied). Because many local minima were found, various other more powerful numerical methods were tested including another deterministic method: (Interior Point IP) and two stochastic methods: (Simulated Annealing SA and the Greedy G Algorithm). Also, several combinations of these methods were tested.

For the deterministic methods (LM and IP), a group of initial guesses within an ample range of values expected for each parameter were combined using nested cycles. Each initial guess was optimized obtaining several hundred optimum solutions. Sometimes equal solutions were obtained starting from different initial guesses. Parameters obtained in successful optimizations were stored in a matrix for later comparison to choose the minimum value of the objective function among several minima. IP proved to be the most effective because it achieved the most optimum values for the parameters while respecting the inherent constraints of the problem. LM cannot use constraints or set bounds, and many times produced unfeasible values for some parameters. Compared to stochastic methods, deterministic methods find significantly better solutions when given a good set of initial values.

The stochastic methods tested bounded values of the parameters. The stochastic methods were less effective because they usually produced results that could not be improved easily by random testing. Additionally, it took much longer computational time for the stochastic methods to produce a solution as optimal as the deterministic methods. The results were significantly different from among the methods. For instance, for a set of data the minimum IP objective function was 0.019 whereas, it was 0.045 for SA. Detailed information and results of this optimization process are described elsewhere.[156]

Because the parameter search was extensive, there is a good chance that the reported parameters are near the global minimum of the objective function. In addition

to *R*, Models 1 and 2 were statistically compared on the basis of the highest F_c as proposed by Froment and Bischof:[157]

$$F_c = \frac{\sum_{i=1}^{n} \frac{\hat{y}_i^{\,2}}{p}}{\sum_{i-1}^{n} \frac{(y_i - \hat{y}_i)^2}{n-p}} \tag{34}$$

where

$\hat{y}_i$ = estimated value of dependent value

p = number of parameters in the model

n = number of experiments

y_i = measured data

Results and discussion

Through lime pretreatment, lignin, cellulose and hemicellulose degradation are direct functions of time and temperature, and are enhanced with aeration. Under the alkaline conditions of long-term lime pretreatment, a strong correlation between lignin and xylan degradation has been demonstrated.[163] Cellulose degradation was minimal; thus, lime pretreatment is selective for lignin and hemicellulose.[163]

In both oxidative and non-oxidative modes, as shown by low *R* for lignin, glucan, and xylan, model fit was very good using both Models 1 and 2 (Table 25); thus, on the basis of this criterion, either model is acceptable. Figure 47 shows very good data fit for Model 1 (data fit for Model 2 was very similar.)[121]

Table 25. F_c for Models 1 and 2

Model	F_c		Sum of residuals	
	Two moieties (Model 1)	Three moieties (Model 2)	Two moieties (Model 1)	Three moieties (Model 2)
Lignin oxidative	14,100	8530	0.0074	0.0069
Glucan oxidative	10,400	5190	0.0133	0.0153
Xylan oxidative	8590	4460	0.0194	0.0208
Glucan non-oxidative	720,000	12,000	0.0072	0.0086
Xylan oxidative	7210	4190	0.0163	0.0156
Xylan non-oxidative	10,500	6000	0.0135	0.0135

Tables 26, 27, and 28 summarize parameters with their corresponding confidence intervals and statistic indicators for Models 1 and 2 applied to oxidative and non-oxidative degradations of lignin, glucan, and xylan, respectively. All confidence intervals are wide, particularly for frequency factors, which span the unacceptable value of zero. Parameter confidence intervals were wider for parameters in Model 2 than in Model 1. The optimization techniques that searched for parameters were systematically and extensively addressed, so it is unlikely that further improvement is possible by selecting alternative optimization techniques. It is possible that improvement could result from specialized experimental designs directed to narrowing confidence intervals for parameters (i.e., D-optimal experimental design[168]). This could be explored in future work.

In Model 2, confidence intervals for parameters were wider than in Model 1. Further, in Model 2 Y_{if0} was consistently low indicating that a third moiety is not

necessary. The most critical case is given by $Y_{Xf0} = 2.22\times10^{-14}$ (Table 28). Because, Model 1 has fewer parameters, it consistently has a higher F_c than Model 2 (Table 25). The F_c criterion rewards models for parsimony (simplicity and fewer parameters). Considering these results, Model 1 was always selected for the degradation of lignin, glucan, and xylan in both oxidative and non-oxidative modes.

Because the widest confidence intervals were observed for a_{ij}, the impact of this parameter on Model-1 predictability is discussed next taking F_c as the response variable for this analysis. Decreasing F_c (the reported value is optimal, increasing is not possible unless through further optimization) implies deterioration in model fit.

For oxidative and non-oxidative lignin degradation, 50% change in both a_{Lf} and a_{Ls} resulted in 60 to 100% reduction in F_c; thus, lignin degradation is sensitive to frequency factors for both lignin moieties.

Conversely, for oxidative glucan degradation, changes up to 100% in a_{Gs} and making $a_{Gs} = 0$ produced < 1% change in the corresponding F_c; however, 50% change in a_{Gf} resulted in about 90% change in F_c. In non-oxidative glucan degradation, changes up to 100% in a_{Gf} and making $a_{Gf} = 0$gave < 1% change in F_c; however, 50% change in a_{Gs} gave close to 90% change in F_c. According to these results, glucan degradation in both oxidative and non-oxidative modes may be modeled taking into account just one moiety with constant degradation speed. Nevertheless, both moieties were considered because they are more reasonably explained from the reactions viewpoint. When changing oxidative and non-oxidative xylan degradation a_{Xf} up to 90% F_c changed < 1%, but 50% change in a_{Xs} gave close to 90% change in F_c; nevertheless, making $a_{Xf} = 0$ produced an

F_c decrease of about 90%; thus, this factor is important in the model and must be taken into account.

Lignin degradation. For oxidative pretreatment, the highest observed delignification, was obtained for the pretreatment at 65°C and 12 weeks (0.71 g lignin degraded/g lignin in raw biomass) and was little less than twice the highest delignification in non-oxidative pretreatment obtained for the same temperature and time (0.40 g lignin degraded/g lignin in raw biomass). Fast degrading fractions of lignin (Y_{Lf0}) are summarized in Table 26 and illustrated in Figures 48a and 48b for oxidative and non-oxidative pretreatments respectively. Y_{Lf0} is higher in the oxidative mode than in the non-oxidative mode.

Additionally, with 12 weeks of pretreatment at 35°C, all of Y_{Lf0} was removed for both oxidative and non-oxidative modes, whereas Y_{Ls0} degraded much more slowly such that at 12 weeks and 65°C approximately 40% and 75% of Y_{Ls0} (oxidative and non-oxidative respectively) still remained showing that Y_{Ls0} is much harder to attack in the absence of oxygen. As a result of reactions with oxygen, lignin becomes more hydrophilic thereby it is easier to remove from the pulp. Oxygen reacts with phenolic structures; however, not all of these structures are reactive[100] ensuing lignin moieties that are not degraded even for pretreatments lasting 12 weeks. An extensive list of lignin degradation products obtained in oxidative alkaline media can be found elsewhere.[106]

Table 26. Parameter estimates and confidence intervals for lignin models ($\alpha = 0.05$).

Parameter	Units	Model 1[a]	Model 2[b]	Model 1[c]	Model 2[d]
		Oxidative pretreatment		Non-oxidative pretreatment	
$Y_{Lf0} \pm$ CI	$\frac{\text{g lignin remaining}}{\text{g lignin in raw biomass}}$	0.235 ± 0.0365	0.0411 ± 0.0626	0.199 ± 0.0360	0.0681 ± 0.100
$a_{Lf} \pm$ CI	min^{-1}	2060 ± 18230	$101 \pm 9.56\times10^{21}$	$8.28\times10^{5} \pm 5.64\times10^{6}$	$100 \pm 3.99\times10^{8}$
$E_{Lf} \pm$ CI	kJ/mol	20.6 ± 11.1	$0.050 \pm 9.56\times10^{21}$	36.2 ± 17.3	$4.90 \pm 9.85\times10^{6}$
$Y_{Lm0} \pm$ CI	$\frac{\text{g lignin remaining}}{\text{g lignin in raw biomass}}$	–	0.204 ± 0.062	–	0.150 ± 0.0988
$a_{Lm} \pm$ CI	min^{-1}	–	4758 ± 18065	–	$5.87\times10^{5} \pm 4.43\times10^{6}$
$E_{Lm} \pm$ CI	kJ/mol	–	23.5 ± 9.98	–	37.0 ± 19.6
$Y_{Ls0} \pm$ CI	$\frac{\text{g lignin remaining}}{\text{g lignin in raw biomass}}$	$0.765 \pm$ NA[e]	$0.755 \pm$ NA[f]	$0.801 \pm$ NA[e]	$0.781 \pm$ NA[f]
$a_{Ls} \pm$ CI	min^{-1}	714 ± 725	699 ± 1266	$4.87\times10^{5} \pm 4.98\times10^{6}$	$5.07\times10^{5} \pm 7.38\times10^{6}$
$E_{Ls} \pm$ CI	kJ/mol	25.6 ± 3.39	25.6 ± 5.14	47.6 ± 29.0	48.3 ± 41.9

[a] 95% confidence interval half-widths for the predicted variable (Y_L) varied between 0 and 0.02
[b] 95% confidence interval half-widths for the predicted variable (Y_L) varied between 0 and 0.02
[c] 95% confidence interval half-widths for the predicted variable (Y_L) varied between 0 and 0.03
[d] 95% confidence interval half-widths for the predicted variable (Y_L) varied between 0 and 0.26
[e] Calculated as $1-Y_{Lf0}$
[f] Calculated as $1- Y_{Lf0}- Y_{Lm0}$
CI: Confidence intervals

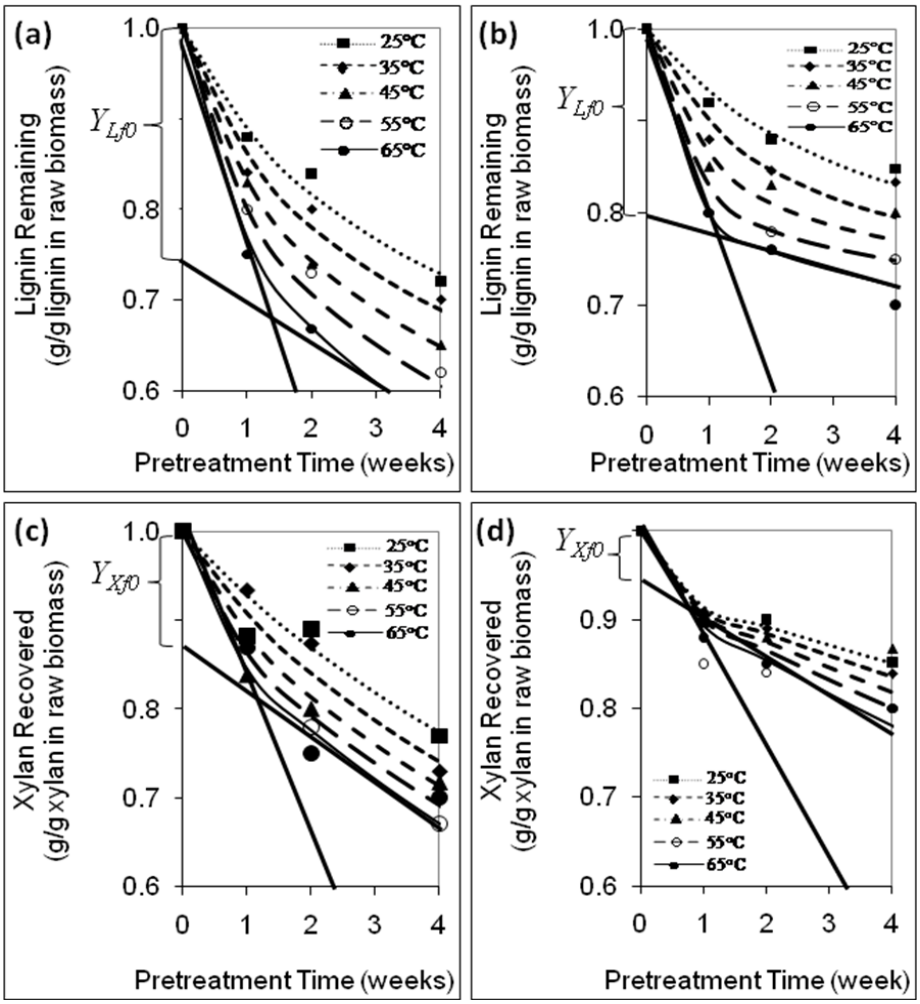

Figure 48. Fast degrading (a) lignin oxidative, (b) lignin non-oxidative, (c) glucan oxidative, (d) glucan non-oxidative

Furthermore, reaction rate constants k_{Lf} for oxidative mode resulted up to 1.5 times those in non-oxidative mode, and k_{Ls} for oxidative mode are from 3 to 10 times those in non- oxidative mode; giving more evidence that the effect of oxygen is specifically directed to hard-to-degrade lignin moieties.

The enhancing effect of oxygen on delignification is notable considering that in oxidative long-term pretreatment, the oxygen concentration in water does not exceed

2.56×10^{-9} molal (upper boundary estimated as suggested by Tromans[169] as function of pH and temperature). For both oxidative and non-oxidative pretreatments, reaction rate constants k_{Lf} are higher than the corresponding k_{Ls} (as expected). In the oxidative mode, this is by factors ranging between 17 and 20 with the higher factors corresponding to the lower temperatures. In the non-oxidative mode, the factors range between 90 and 150 and in both cases, the higher factors correspond to the lower temperatures.

Interestingly, the change from the rapid initial phase to the slower final phase occurs simultaneously for both lignin and xylan, and for both oxidative and non-oxidative pretreatments (at about 1 week of pretreatment as seen in Figure 48), which shows that they are chemically related.[163]

Glucan degradation. For oxidative pretreatment, the highest observed glucan degradation was obtained for the pretreatment at 65°C and 12 weeks (0.40 g glucan degraded/g glucan in raw biomass) which is twice the highest glucan degradation in non-oxidative pretreatment obtained for the same temperature and time (0.20 g glucan degraded/g glucan in raw biomass); thus, the presence of oxygen triggers glucan degradation which is expectable in accordance to previous research.[100] An extensive list of glucan degradation products obtained in oxidative alkaline media can be found elsewhere.[96, 97] Glucan fast degrading fractions are not evidently separable in the plots because they are very small. In the oxidative pretreatment, according to Model 2, Y_{Gm0} = 0.0037 g glucan remaining/g glucan in raw biomass.

Table 27. Parameter estimates and confidence intervals for glucan models ($\alpha = 0.05$).

Parameter	Units	Model 1[a]	Model 2[b]	Model 1[c]	Model 2[d]
		Oxidative pretreatment		Non-oxidative pretreatment	
$Y_{Gf0} \pm$ CI	$\frac{\text{g glucan remaining}}{\text{g glucan in raw biomass}}$	0.544 ± 0.492	$0.0037 \pm$ NA[f]	0.0127 ± 0.845	$0.0076 \pm$ NA[f]
$a_{Gf} \pm$ CI	min^{-1}	$53212 \pm 2.05\times10^{5}$	$9.02\times10^{11} \pm 1.01\times10^{23}$	$511 \pm 8.53\times10^{-3}$	$3.93\times10^{11} \pm 1.27\times10^{28}$
$E_{Gf} \pm$ CI	kJ/mol	36.7 ± 10.9	$53.0 \pm 1.01\times10^{23}$	25.6 ± 1131	$20.0 \pm 1.27\times10^{28}$
$Y_{Gm0} \pm$ CI	$\frac{\text{g glucan remaining}}{\text{g glucan in raw biomass}}$	–	0.430 ± 2.48	–	0.370 ± 867
$a_{Gm} \pm$ CI	min^{-1}	–	$1.84\times10^{5} \pm 4.32\times10^{6}$	–	$2.74\times10^{11} \pm .90\times10^{13}$
$E_{Gm} \pm$ CI	kJ/mol	–	39.5 ± 56.3	–	81.9 ± 285
$Y_{Gs0} \pm$ CI	$\frac{\text{g glucan remaining}}{\text{g glucan in raw biomass}}$	$0.456 \pm$ NA[e]	0.566 ± 2.48	$0.987 \pm$ NA[e]	0.622 ± 3.13
$a_{Gs} \pm$ CI	min^{-1}	$75496 \pm 1.91\times10^{11}$	65.4 ± 13614	$1.51\times10^{6} \pm 4.27\times10^{7}$	61.3 ± 4446
$E_{Gs} \pm$ CI	kJ/mol	$83.0 \pm 1.39\times10^{7}$	25.8 ± 445	49.8 ± 105	23.0 ± 176

[a] 95% confidence interval half-widths for the predicted variable (Y_G) varied between 0 and 0.04
[b] 95% confidence interval half-widths for the predicted variable (Y_G) varied between 0 and 0.71
[c] 95% confidence interval half-widths for the predicted variable (Y_G) varied between 0 and 0.25
[d] 95% confidence interval half-widths for the predicted variable (Y_G) varied between 0 and 161
[e] Calculated as $1-Y_{Gf0}$
[f] Calculated as $1- Y_{Gm0}- Y_{Gs0}$
CI: Confidence intervals

In the non-oxidative mode, according to Model 1, Y_{Gs0} = 0.0127 g glucan remaining/g glucan in raw biomass and according to Model 2 Y_{Gm0} = 0.0076 g glucan remaining/g glucan in raw biomass (Table 27); thus, as soon as pretreatment starts, some peeling degradation of glucan takes place very rapidly, but stopping reactions follow very soon and degradation of remaining glucan will depend on the generation of new accessible reducing end groups via mid-chain alkaline scission of chemically stopped accessible material which is more likely in oxidative mode. Fast-degrading glucan former reacts as a function of time and temperature, and is the dominant contribution to the degradation process. In contrast, the slow-degrading glucan fraction remains essentially constant at Y_{Gs0} = 0.46 g glucan/g glucan in raw biomass.

k_{Gf} is 1×10^{7} to 9×10^{7} times greater than the corresponding k_{Gs}, depending on temperature. Of the initial medium-degrading glucan (Y_{Gm0} = 0.54 g glucan/g glucan in raw biomass), a maximum of 0.40 g glucan are degraded, leaving the difference of 0.14 g glucan unchanged. Therefore, the lowest observed glucan yield was 0.14 + 0.46 = 0.60 g glucan re maining/g glucan in raw biomass, which was observed in the most aggressive pretreatment (65°C and 12 weeks).

Although the radical reactions that occur with oxygen are largely responsible for delignification, they also degrade cellulose. Oxygen-based radicals, especially hydroxyl radicals, can oxidize cellulose hydroxyl groups to ketones. Under the strongly basic conditions used in lime pretreatment, these compounds undergo reverse aldol reactions leading to cellulose cleavage, thereby increasing the glucan degradation rate.[104]

In the non-oxidative mode, k_{Gf} is between 1 and 10 times greater than the corresponding k_{Gs}, and the fraction of fast-degrading glucan (Y_{Gf0}) is only 0.013 g glucan/g glucan in raw biomass. In the most aggressive pretreatment (65°C for 12 weeks), this fraction does not degrade completely, but only to a minimum of 0.006 g glucan/g glucan in raw biomass (47%). Consequently, in alkaline media, there is a mechanism responsible for cellulose degradation that does not require hydroxyl radicals. This is the *peeling reaction*, in which the cellulose chain is progressively shortened by the loss of single glucose units from one end of the chain. In competing *stopping* reactions that occur at lower rates, the reducing glucose groups are converted to carboxylic acid end groups that render the cellulose stable in alkaline media.[170] An additional proposed explanation to the fact that glucan dissolution is incomplete is that $CaCO_3$ deposits (possibly obtained from CO_2 that results from degradation and reacts with $Ca(OH)_2$) prevent the occurrence of peeling reactions; thus glucan peeling only occurs to a limited extent.[85]

Degradation of slow glucan may also benefit from stopping reactions and salt deposits. The minimum glucan yield for non-oxidative pretreatment was 0.69 g glucan remaining/g glucan in raw biomass for the most aggressive pretreatment (12 weeks at 65°C).

Xylan degradation. For oxidative pretreatment, the highest observed xylan degradation was obtained for the pretreatment at 65°C and 12 weeks (0.63 g xylan degraded/g xylan in raw biomass) which is about 1.5 times the highest xylan degradation

Table 28. Parameter estimates and confidence intervals for xylan models ($\alpha = 0.05$).

Parameter	Units	Model 1[a]	Model 2[b]	Model 1[c]	Model 2[d]
		Oxidative pretreatment		Non-oxidative pretreatment	
$Y_{Xf0} \pm$ CI	$\frac{\text{g xylan remaining}}{\text{g xylan in raw biomass}}$	0.111± NA[e]	0.107 ± NA[e]	0.0647 ±0.0193	2.22×10^{-14}± 0.87
$a_{Xf} \pm$ CI	min^{-1}	4934± 53318	$8.19\times10^{5}\pm1.74\times10^{7}$	1212± 1.33×10^{23}	136 ± 2.69×10^{37}
$E_{Xf} \pm$ CI	kJ/mol	23.0 ± 27.3	36.2 ± 53.8	7.64 ± 1.33×10^{23}	11.4 ± 2.69×10^{37}
Y_{Xm0}[a] $\pm$ CI	$\frac{\text{g xylan remaining}}{\text{g xylan in raw biomass}}$	–	0.0207 ± 2.16×10^{5}	–	0.935± NA[g]
$a_{Xm} \pm$ CI	min^{-1}	–	3.23 ± 3.07×10^{6}	–	5.32 ± 12.5
$E_{Xm} \pm$ CI	kJ/mol	–	10.7 ± 2.53×10^{6}	–	13.4 ± 4.45
$Y_{Xs0} \pm$ CI	$\frac{\text{g xylan remaining}}{\text{g xylan in raw biomass}}$	0.889 ± 0.0588	0.873 ± 2.16×10^{5}	0.935± NA[f]	0.0647 ± 1.29×10^{13}
$a_{Xs} \pm$ CI	min^{-1}	2.69 ± 3.35	2.69 ± 6.09×10^{5}	5.35 ± 6.39	1212 ± 2.29×10^{22}
$E_{Xs} \pm$ CI	kJ/mol	10.2 ± 2.83	10.2 ± 6.03×10^{5}	13.4 ± 3.23	7.64 ± 2.13×10^{22}

[a] 95% confidence interval half-widths for the predicted variable (Y_X) varied between 0 and 0.03
[b] 95% confidence interval half-widths for the predicted variable (Y_X) varied between 0 and 648
[c] 95% confidence interval half-widths for the predicted variable (Y_X) varied between 0 and 0.02
[d] 95% confidence interval half-widths for the predicted variable (Y_X) varied between 0 and 1.98×10^{14}
[e] Calculated as $1-Y_{Xs0}$
[f] Calculated as $1- Y_{Xf0}- Y_{Xm0}$
[g] Calculated as $1- Y_{Xf0}- Y_{Xs0}$
CI: Confidence intervals

in non-oxidative pretreatment obtained for the same temperature and time (0.40 g xylan degraded/g xylan in raw biomass); thus, the presence of oxygen triggers xylan degradation which is expectable in accordance to previous research.[171] Nevertheless, the effect of oxygen seems more notable on lignin and glucan degradation than it is on xylan degradation.[163] An extensive list of xylan degradation products obtained in oxidative alkaline media can be found elsewhere.[96, 97]

For the oxidative pretreatment, the initial fraction of fast-degrading xylan (Y_{Xf0} = 0.11 g xylan/g xylan in raw biomass, see Figure 48c and Table 28) completely dissolved within 8 weeks of pretreatment depending of temperature (e.g., only 4 weeks of pretreatment if at 65°C). In this stage, radical attack at the glycosidic linkage occurs, after which the degradation is dominated by a depolymerization reaction.[171] Concurrently, gradual dissolution of slow-degrading xylan occurs and for the longest time and temperature the maximum extent of degradation of this xylan fraction is about 60%. In this stage, a competition between depolymerization and recombination reactions occurs.[171]

The initial fraction of fast-degrading xylan (Y_{Xf0}) is only 0.065 g xylan/g xylan in raw biomass (Figure 48d and Table 28) and all of this xylan dissolves within the first week of pretreatment regardless of temperature. In this stage, the reducing xylose group is easily isomerized and removed by β-elimination, which leads to reducing galacturonic acid end groups. This is converted to other groups that are very stable in alkaline media.[166]

In the oxidative mode, k_{Xf} is 10 to 20 times greater than the corresponding k_{Xs} with the largest differences observed at the highest temperatures. In non-oxidative mode, k_{Xf} is 1800 to 2400 times greater than the corresponding k_{Gs} with the largest differences corresponding to the lower temperatures. Not only k_{Xf} and k_{Xs} are significantly closer in magnitude in the oxidative mode than in the non-oxidative mode but also k_{Xs} oxidative > k_{Xs} non-oxidative; thus, the main effects of oxygen are directed to hard-to-degrade xylan moieties (slow-xylan) as it is the case for lignin and glucan.

Additionally, the effect of temperature is more noticeable in the oxidative mode than in the non-oxidative mode because differences in k_{Xf} and k_{Xs} increase in the same direction as temperature for oxidative mode and in opposite direction for non-oxidative mode.

The degradation rate of slow xylan depends on temperature and time. It reaches a maximum degradation of about 40% and occurs upon prolonged alkaline treatment. This degradation starts with a rapid peeling that arises because many galacturonic groups form and then are lost. This peeling stops when a xylose group with a 4-O-methylgucuronic acid substituent is liberated.[166]

Model assessment

Models 1 and 2 for lignin, glucan and xylan were assessed through calculation of R and F_c (Table 25) and confidence intervals for the predicted variable (footnotes on Tables 26, 27, and 28).

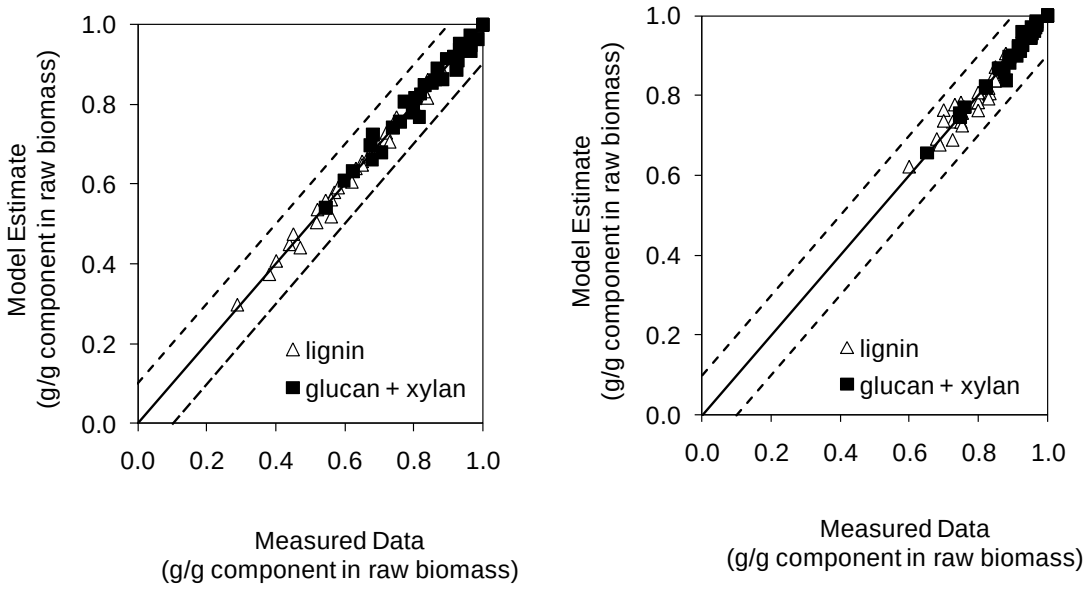

Figure 49. Model assessment. Dotted lines describe 95% prediction intervals (a) non-oxidative (b) oxidative.

All of these indicators showed Models 1 and 2 decisively commendable, particularly Model-1, with the only drawback of wide confidence intervals for parameters. Also, Figure 49 shows that variability of Model-1 data is within 95% prediction intervals.

Selectivity

Selectivity measures the ability of pretreatment to degrade lignin while retaining carbohydrates. Glucan and xylan selectivities were calculated in two forms: *differential* defined as the ratio of lignin degradation rate to carbohydrate degradation rate, and

integral defined as the ratio of lignin removed to carbohydrate removed. The obtained results follow:

Differential selectivity is defined as follows:

$$S_{dG} \equiv \frac{dY_L/dt}{dY_G/dt} = \frac{a_{Lf}\exp\left(-\frac{E_{Lf}}{RT}\right)Y_{Lf} + a_{Ls}\exp\left(-\frac{E_{Ls}}{RT}\right)Y_{Ls}}{a_{Gf}\exp\left(-\frac{E_{Gf}}{RT}\right)Y_{Gf} + a_{Gs}\exp\left(-\frac{E_{Gs}}{RT}\right)Y_{Gs}}$$

(35)

$$S_{dX} \equiv \frac{dY_L/dt}{dY_X/dt} = \frac{a_{Lf}\exp\left(-\frac{E_{Lf}}{RT}\right)Y_{Lf} + a_{Ls}\exp\left(-\frac{E_{Ls}}{RT}\right)Y_{Ls}}{a_{Xf}\exp\left(-\frac{E_{Xf}}{RT}\right)Y_{Xf} + a_{Xs}\exp\left(-\frac{E_{Xs}}{RT}\right)Y_{Xs}} \quad (36)$$

The highest differential glucan selectivities for both oxidative and non-oxidative pretreatment are observed at the beginning of pretreatment, for high lignin content, and at low temperatures. However, as lignin content decreases to ≤ 0.70 g lignin remaining/g lignin in raw biomass (0.80 for non-oxidative pretreatment) and pretreatment time increases to ≥ 8 weeks, the differences in glucan selectivity observed for different temperatures become very small (Figure 50).

For oxidative pretreatment, the highest glucan selectivity is ~13 g lignin removed/g glucan removed and the lowest is ~1.2 g lignin removed/g glucan removed. For non-oxidative pretreatment, the highest selectivity is ~27 g lignin removed/g glucan

removed and selectivity decreases much faster and reaches a minimum of ~0.80 g lignin removed/g glucan removed at 1 week of pretreatment and 65°C.

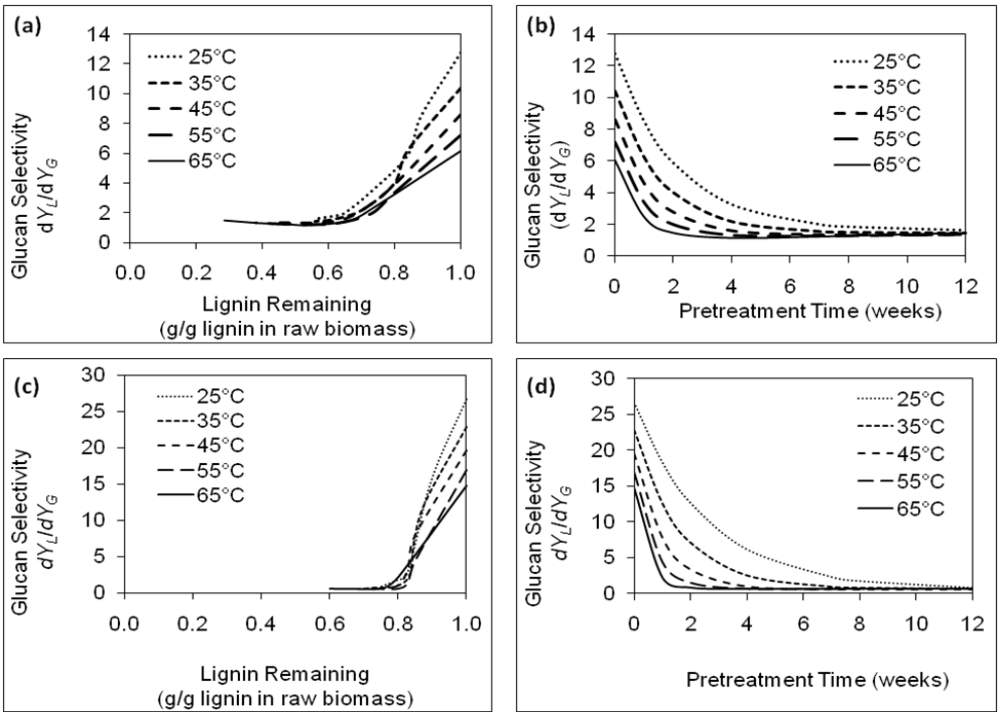

Figure 50. Differential selectivity of glucan (a & b) oxidative (c & d) non-oxidative.

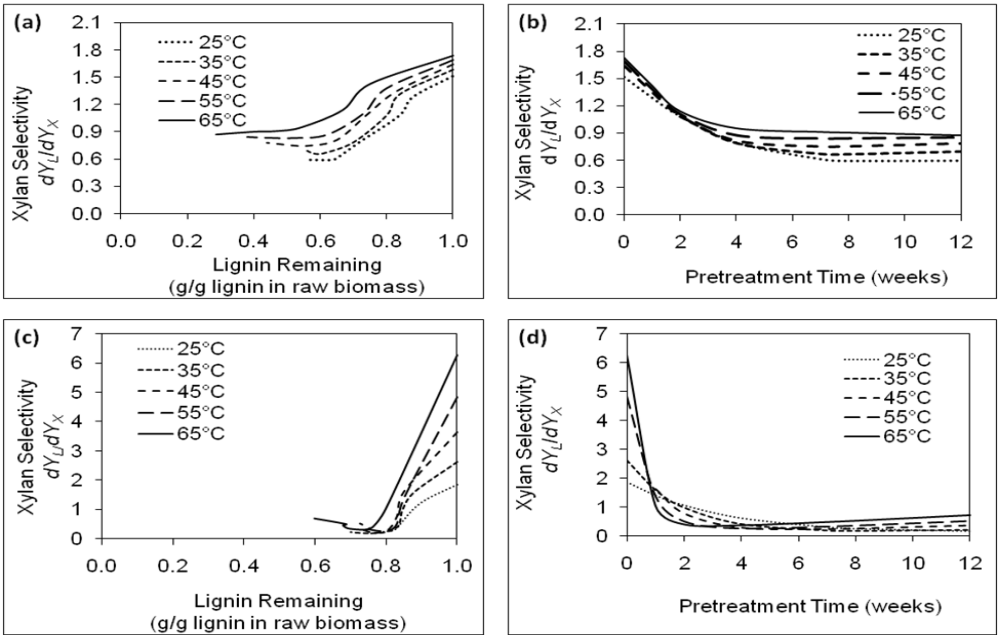

Figure 51. Differential selectivity of xylan (a & b) oxidative (c & d) non-oxidative.

The results for xylan selectivity (Figure 51) are interestingly opposed to those of glucan selectivity regarding pretreatment temperature, i.e., xylan selectivity is higher at higher temperatures. However, similar to glucan selectivity, higher xylan selectivities are observed at the beginning of pretreatment, when lignin contents are high. A minimum selectivity of ~0.70 g lignin removed/g xylan removed is observed at 1 week of pretreatment with very slow increase after that, particularly at 65°C.

At all pretreatment conditions, xylan selectivity is much lower than the corresponding glucan selectivity. Lignin and xylan are chemically bonded;[125] thus, it is not surprising that as lignin degrade so does xylan while glucan degradation takes place independently and much more slowly.

In oxidative pretreatment, the highest xylan selectivity observed (~2 g lignin removed/g xylan removed) is about 6 times smaller than the glucan selectivity for the

same pretreatment conditions. In non-oxidative pretreatment, the highest xylan selectivity is ~6 g lignin removed/g xylan removed, which is about 4.5 times smaller than glucan selectivity at the same pretreatment conditions.

Xylan selectivity does not strongly depend on temperature. In non-oxidative pretreatment, as lignin content decreases to ≤ 0.8 g lignin remaining/g lignin in raw biomass and for pretreatment times ≥ 4 weeks, the differences in selectivity observed for different pretreatment temperatures are negligible. The smallest xylan selectivities were ~0.6 g lignin removed/g xylan removed for the oxidative pretreatment and ~0.2 g lignin removed/g xylan removed for non-oxidative pretreatment.

Integral selectivity is defined as follows:

$$S_G \equiv \frac{1-Y_L}{1-Y_G} = \frac{1-Y_{Lf0}\exp\left(-a_{Lf}\exp\left(-\frac{E_{Lf}}{RT}\right)t\right)-Y_{Ls0}\exp\left(-a_{Ls}\exp\left(-\frac{E_{Ls}}{RT}\right)t\right)}{1-Y_{Gf0}\exp\left(-a_{Gf}\exp\left(-\frac{E_{Gf}}{RT}\right)t\right)-Y_{Gs0}\exp\left(-a_{Gs}\exp\left(-\frac{E_{Gs}}{RT}\right)t\right)} \quad (37)$$

$$S_X \equiv \frac{1-Y_L}{1-Y_X} = \frac{1-Y_{Lf0}\exp\left(-a_{Lf}\exp\left(-\frac{E_{Lf}}{RT}\right)t\right)-Y_{Ls0}\exp\left(-a_{Ls}\exp\left(-\frac{E_{Ls}}{RT}\right)t\right)}{1-Y_{Xf0}\exp\left(-a_{Xf}\exp\left(-\frac{E_{Xf}}{RT}\right)t\right)-Y_{Gs0}\exp\left(-a_{Xs}\exp\left(-\frac{E_{Xs}}{RT}\right)t\right)} \quad (38)$$

Oxidative and non-oxidative integral selectivity for glucan and xylan showed the same tendencies as the corresponding differential selectivities; however, differences in selectivity observed for different temperatures are more noticeable.

For oxidative pretreatment, integral and differential selectivity were very similar for the same pretreatment conditions; however, in a few cases, glucan integral selectivity was up to 2 times higher than the corresponding glucan differential selectivity and xylan integral selectivity was about 50% higher than the corresponding xylan differential selectivity. In non-oxidative pretreatment, integral and differential selectivities were very similar for most cases, except for a few cases in glucan selectivity where differences were more important (i.e., at 25°C and 12 weeks, glucan integral selectivity was ~7 times higher than the corresponding differential selectivity).

Conclusions

Widely accepted kinetic models for alkaline delignification use a standard power rate equation that includes temperature and pretreatment time as control variables, and consider two or three moieties of lignin or carbohydrates. In this work, these models have been successfully applied to long-term lime pretreatment of poplar wood. On the basis of better statistical results, two moieties were preferred. Because of important differences in delignification mechanisms with and without air, the model parameters were significantly different.

For oxidative delignification, all activation energies ranged from 20 to 26 kJ/mol. For non-oxidative delignification, they ranged from 35 to 50 kJ/mol. For both oxidative and non-oxidative glucan degradation, the activation energies varied from 25 to 84

kJ/mol, whereas for xylan degradation, they were much lower varying from 7 to 24 kJ/mol.

These models were used to calculate glucan and xylan selectivity in two fashions: differential and integral. These show similar tendencies, but integral selectivity was higher than differential selectivity. For both oxidative and non-oxidative pretreatment, glucan selectivities are higher at high temperature, high lignin content, and at the beginning of pretreatment. Also, glucan selectivities were much higher than xylan selectivities. Very noticeable cases are observed at 25°C, 0 weeks, and oxidative pretreatment, where glucan selectivity was about 15 times than the corresponding xylan selectivity. For the same temperature, time, and non-oxidative pretreatment, glucan selectivity was about 7 times higher than the corresponding xylan selectivity.

Xylan selectivities were higher at lower temperatures, high lignin content, and at the beginning of pretreatment. Xylan integral and differential selectivities were more similar than for glucan selectivities.

LIME PRETREATMENT CONDITIONS TO OBTAIN TARGET COMPOSITIONAL CHANGES

Synopsis

Lime pretreatment is an effective way to significantly affect composition of poplar wood in any of four modes: long-term non-oxidative (LTO), long-term oxidative (LTN), short-term constant pressure (CP), and short-term varying pressure (VP). Using non-linear optimization techniques with non-linear constrains, pretreatment conditions were identified to obtain a target lignin yield (Y_L) varying between 0.2 to 0.8 g lignin/g lignin in raw biomass with maximum glucan yield (Y_G) or selectivity. For all lignin yield targets, VP was identified as the most robust mode of pretreatment that also results in the highest glucan yields.

Introduction

Oxygen delignification of wood is frequently used in pulping and bleaching industries. To significantly increase biomass digestibility, similar techniques may be applied to any type of lignocellulose resulting in compositional changes, mainly partial delignification. The treated therefore more digestible biomass may be used as feedstock for fermentations to fuels and chemicals or as feedstock to produce animal feed.[16]

In lime pretreatment, calcium hydroxide is combined with lignocellulosic feedstocks, water, and possibly air or pressurized oxygen as oxidative agents. The mixture is submitted to temperatures ranging from 25 to 180°C for hours to weeks.[161]

The ranges of the control variables vary widely, so the study of lime pretreatment is divided into four modes: long-term non-oxidative (LTN), long-term oxidative (LTO), constant pressure (CP), and varying pressure (VP). In oxidative alkaline pretreatments, oxygen exposed to pH 11 to 13 undergoes a sequence of reducing reactions to form hydroxyl radicals that attack lignin very effectively. Unfortunately, these radicals also damage cellulose and hemicellulose. In non-oxidative alkaline pretreatment, endwise depolymerization of lignin, cellulose, and hemicellulose occur.

Kinetic models for lignin and carbohydrates degradation are useful for control and design purposes to determine selective pretreatment conditions that remove lignin while preserving carbohydrates. Another important application is to determine pretreatment conditions that obtain target compositional changes in the biomass, which may significantly extend the range of biomass usage.

In this study, pretreatment conditions that maximize glucan yield (Y_G) constrained to a target lignin yield (Y_L) were obtained for poplar wood. The non-linear optimization problem is defined by the following set of equations:

$$\max \quad Y_G = \sum_j Y_{Gj0} \exp(-k_{Gj} P^{\beta Gj} t) \tag{39}$$

Subjected to

$$Y_L = \sum_j Y_{Lj0} \exp(-k_{Lj} P^{\beta Lj} t) = C \tag{40}$$

Model variables and parameters with their corresponding units have already been established for each pretreatment mode.[126, 140, 162] In consecutive optimization runs, C was varied between 0.2 and 0.8 g lignin/g lignin in raw biomass. In essence, the

objective function (Eq. 39) also optimizes selectivity, defined as the ratio of the rate of change in lignin concentration to the rate of change in carbohydrates concentration.[126, 140, 162]

For long-term pretreatment $\beta_{Gj} = \beta_{Lj} = 0$.[126] For CP, [162] P is the reactor total pressure and for VP, P is the ratio of initial mass of oxygen per mass of biomass.[140]

Materials and methods

Poplar wood was obtained from the National Renewable Energy Laboratory (NREL) and prepared by drying and grinding. Detailed description can be found elsewhere.[111] It was used as feedstock for lime pretreatment in four modes: CP, VP, LTO, and LTN. For each of these modes, Table 29 summarizes ranges of pretreatment temperature, oxygen pressure, and pretreatment times.

Table 29. Experimental variable ranges for each pretreatment mode.

Pretreatment mode	Temperature (° C)	Pressure (bar)	Pretreatment time
Long-term oxidative	25-75	Atmospheric[(a)]	1 to 12 weeks
Long-term non-oxidative	25-75	Atmospheric[(b)]	1 to 12 weeks
Short-term constant pressure (CP)	110 - 180	7.9 to 21.7[(c)]	1 to 10 hours
Short-term varying pressure (VP)	140-180	7.9 to 28.5[(d)]	1 to 10 hours

[(a)] Bubbling air
[(b)] No bubbling air
[(c)] Total pressure
[(d)] Oxygen partial pressure

For long-term pretreatments, 15 g of poplar wood were mixed with lime and water and put into reactors made of PVC pipe that were preheated to the desired pretreatment temperature (T). In some cases, saturated air was bubbled into the reactors from the bottom at about 3.5 mL/min. Pretreatment time (t) was several weeks. Details on the pretreatment apparatus and procedures are published elsewhere.[163]

For CP mode, stainless steel reactors were filled with water, poplar wood, and calcium hydroxide ($Ca(OH)_2$), mixed well, and heated to the pretreatment temperature. Afterwards, they were connected to an oxygen line, through which oxygen was applied to the desired total pressure. Because the reactors were connected to the oxygen line at all times during pretreatment, the pressure was constant.[111]

For VP mode, reactors were filled as before, but oxygen was applied only once at the beginning of pretreatment to the desired initial pressure. Details on pretreatment apparatus and procedures are published elsewhere.[111]

Lignin content, carbohydrates content, and other compositional analysis were performed on untreated and pretreated biomass according to NREL analytical procedures.[111, 163] Data on lignin yields (Y_L), glucan yields (Y_G), and xylan yields (Y_X) was used to obtain kinetic parameters to model each pretreatment mode.[126, 140, 162] These models allowed calculation of differential selectivity for glucan (S_{dG}) and xylan (S_{dX}). Solution of the constrained optimization problem of maximizing Y_G subjected to target Y_L varying between 0.2 and 0.8 g lignin/g lignin in raw biomass was obtained through an algorithm (Figure 52) codified in Matlab, using the *fmincon* subroutine. This subroutine

is designed for minimization problems; thus, to address the maximization problem in this study, the objective function was equivalently written as $\min 1/Y_G$

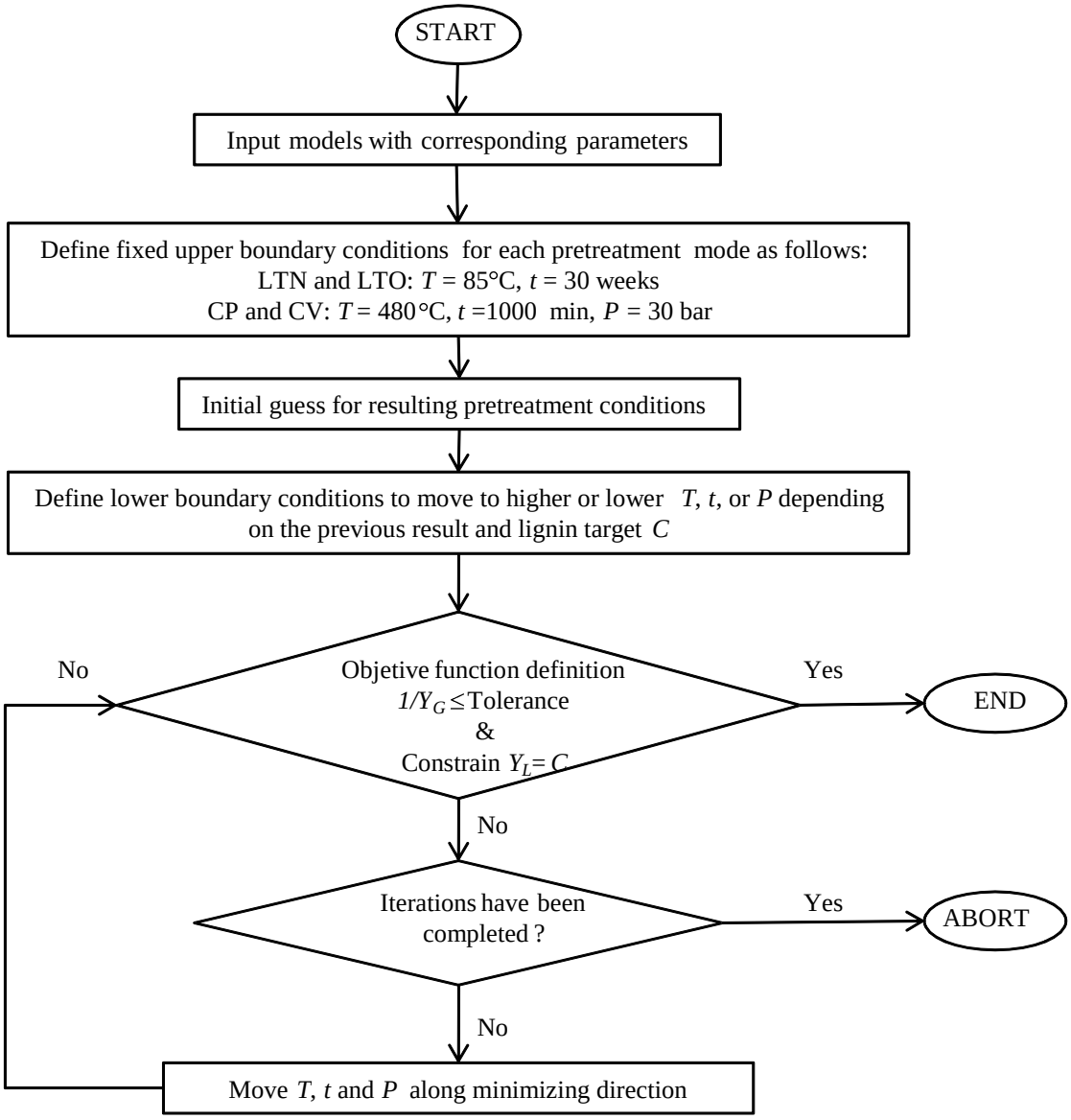

Figure 52. Optimization algorithm to find pretreatment conditions that optimize Y_G for several target Y_L

Results and discussion

Tables 30 to 33 show the reactor conditions that provide the optimal Y_G for a target Y_L. Figures 53 to 56 are contour plots that show how temperature, time, and pressure affect Y_G. Most optima were obtained for pretreatment modes at temperatures close to the lower bound, times close to the upper bound, and if pressurized, high pressures for low lignin targets (Y_L <0.49 g lignin/g lignin in raw biomass) and low pressures for high lignin targets ($Y_L \geq$ 0.5 g lignin/g lignin in raw biomass). Particular features for each pretreatment mode are described next.

Long-term non-oxidative. (Table 30 and Figure 53). Using this pretreatment mode, it is not possible to degrade lignin to Y_L = 0.2 g lignin/g lignin in raw biomass. For Y_L = 0.3, 0.4 and 0.5 g lignin/g lignin in raw biomass, temperatures up to 83°C were required with pretreatment times up to 3 years. In all cases, degradation of glucan and xylan were much more important than lignin degradation; thus, this pretreatment is not selective. Higher lignin targets ($Y_L \geq$0.6 g lignin/g lignin in raw biomass) required shorter pretreatments and lower temperatures, but pretreatments are still very long and less selective compared to other modes of lime pretreatment. Consequently, this mode of pretreatment is not recommended for poplar wood.

Long-term oxidative. (Table 31 and Figure 54). For $Y_L \leq$0.40 g lignin/g lignin in raw biomass, up to 20 weeks of pretreatment were required and maximum Y_G were low

ranging between 0.55 g glucan/g glucan in raw biomass and 0.85 g glucan/g glucan in raw biomass. For $Y_L \geq 0.50$ g lignin/g lignin in raw biomass, 17 weeks of pretreatment or less were required depending on Y_L target, decreasing to a minimum of 2 weeks for Y_L = 0.8 g lignin/g lignin in raw biomass. Maximum $Y_G \geq 0.90$ g glucan/g glucan in raw biomass was obtained in this Y_L range.

Table 30. Descriptive statistics for optimized yields and selectivities in LTN mode

Y_L[(a)]	Y_G[(b)]	Y_X[(c)]	S_{dG}[(d)]	S_{dX}[(e)]	Temperature (°C)	Time (weeks)
0.300	0.068	0.119	1.60	1.19	83	36
0.400	0.171	0.183	0.893	0.902	78	31
0.500	0.299	0.294	0.638	0.719	70	30
0.600	0.491	0.466	0.476	0.507	60	30
0.700	0.737	0.690	0.383	0.400	45	30
0.800	0.979	0.905	2.29	1.74	27	6.3

[(a)]g lignin, glucan or xylan remaining/g lignin, glucan or xylan in raw biomass [(b)]g lignin, glucan or xylan remaining/g lignin, glucan or xylan in raw biomass [(c)] g lignin, glucan or xylan remaining/g lignin, glucan or xylan in raw biomass [(d)] g lignin removed/g glucan or xylan removed [(e)] g lignin removed/g glucan or xylan removed

This mode of pretreatment is more selective than long-term non oxidative and is recommended if reactors are not available that can withstand the more severe conditions of short-term pretreatment.

Short-term constant pressure. (Table 32 and Figure 55). All Y_L targets were achievable showing that CP is very flexible and adaptable. For $Y_L \leq 0.49$ g lignin/g lignin in raw biomass, the highest Y_G were in the range from 0.80 and 0.92 g glucan/g glucan in raw biomass and were preferably obtained at low temperatures, high pressures and long times within the allowed ranges.

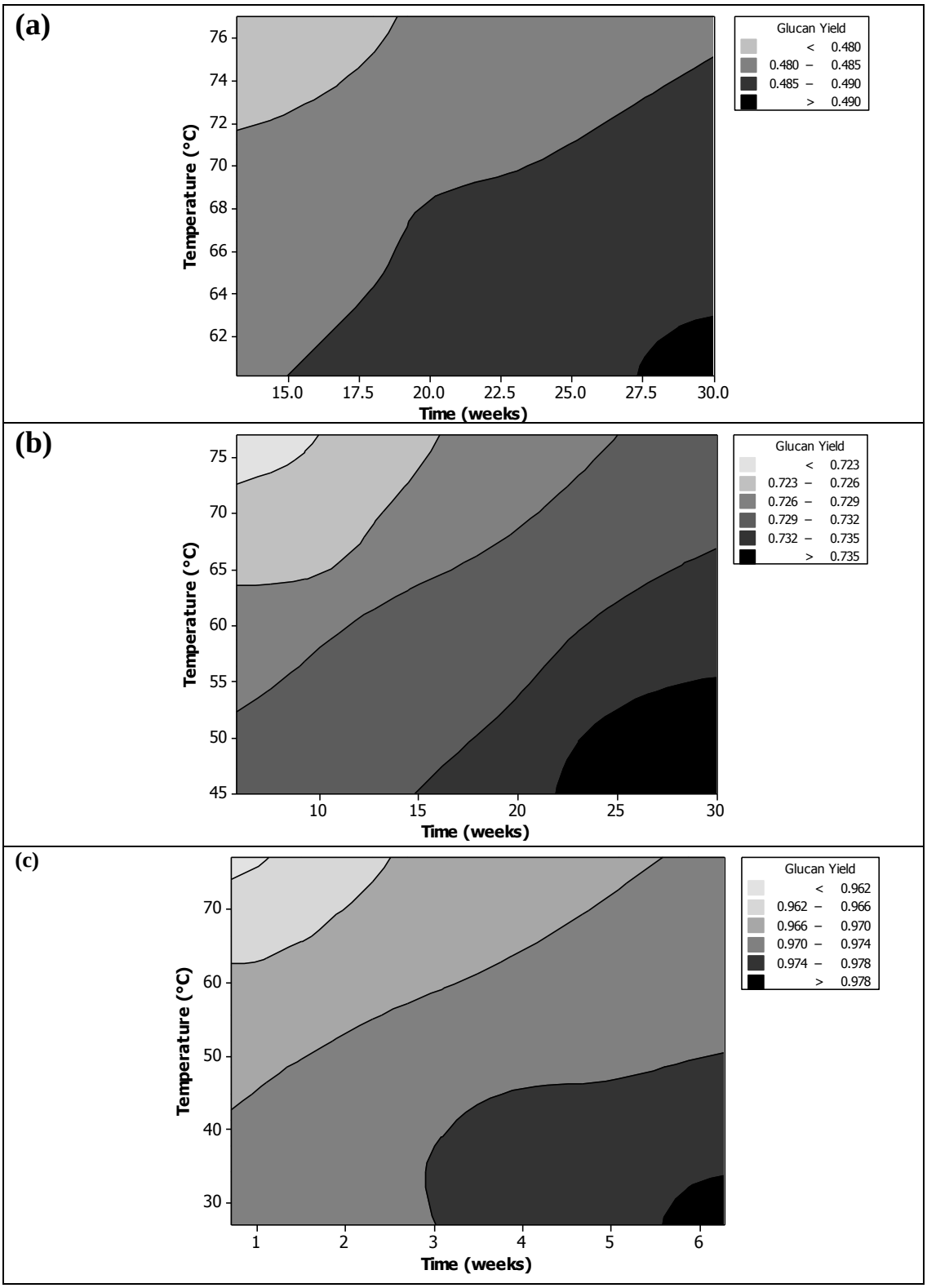

Figure 53. LTN mode. Contour plots for glucan yield (g glucan/g glucan in raw biomass). Target lignin (a) Y_L = 0.6 (b) Y_L = 0.7 (c) Y_L = 0.8 g lignin per g lignin in raw biomass.

For Y_L = 0.50 g lignin/g lignin in raw biomass, low temperature, low pressure and mid time were best and resulted in 0.94 < Y_G < 0.96 g glucan/g glucan in raw biomass.

Table 31. Descriptive statistics for optimized yields and selectivities in LTO mode

Y_L[(a)]	Y_G[(b)]	Y_X[(c)]	S_{dG}[(d)]	S_{dX}[(e)]	Temperature (°C)	Time (weeks)
0.200	0.547	0.320	2.00	0.860	60	20
0.300	0.643	0.441	1.56	0.908	47	20
0.400	0.745	0.547	1.42	0.976	35	20
0.500	0.832	0.646	1.53	1.04	27	17
0.600	0.896	0.738	1.64	1.15	27	10
0.700	0.947	0.817	2.57	1.41	27	1.41
0.800	0.975	0.883	5.10	1.62	27	2

[(a)]g lignin, glucan or xylan remaining/g lignin, glucan or xylan in raw biomass [(b)]g lignin, glucan or xylan remaining/g lignin, glucan or xylan in raw biomass [(c)] g lignin, glucan or xylan remaining/g lignin, glucan or xylan in raw biomass [(d)] g lignin removed/g glucan or xylan removed [(e)] g lignin removed/g glucan or xylan removed

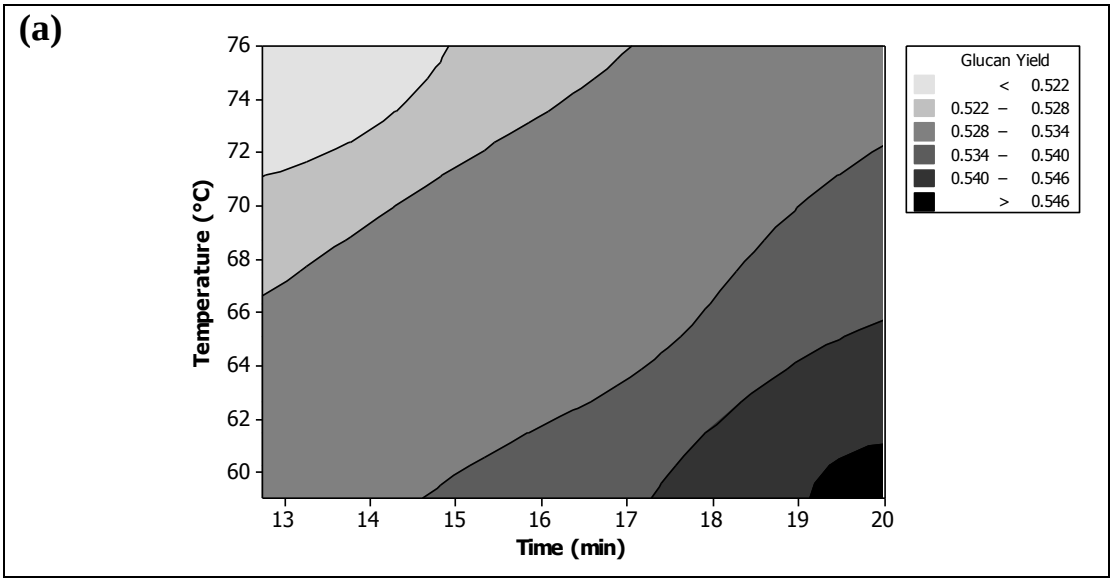

Figure 54. LTO mode. Contour plots for glucan yield (g glucan/g glucan in raw biomass). Target lignin (a) Y_L = 0.2 (b) Y_L = 0.3 (c) Y_L = 0.4 (d) Y_L = 0.5 (e) Y_L = 0.6 (f) Y_L = 0.7 (g) Y_L = 0.8 g lignin/g lignin in raw biomass.

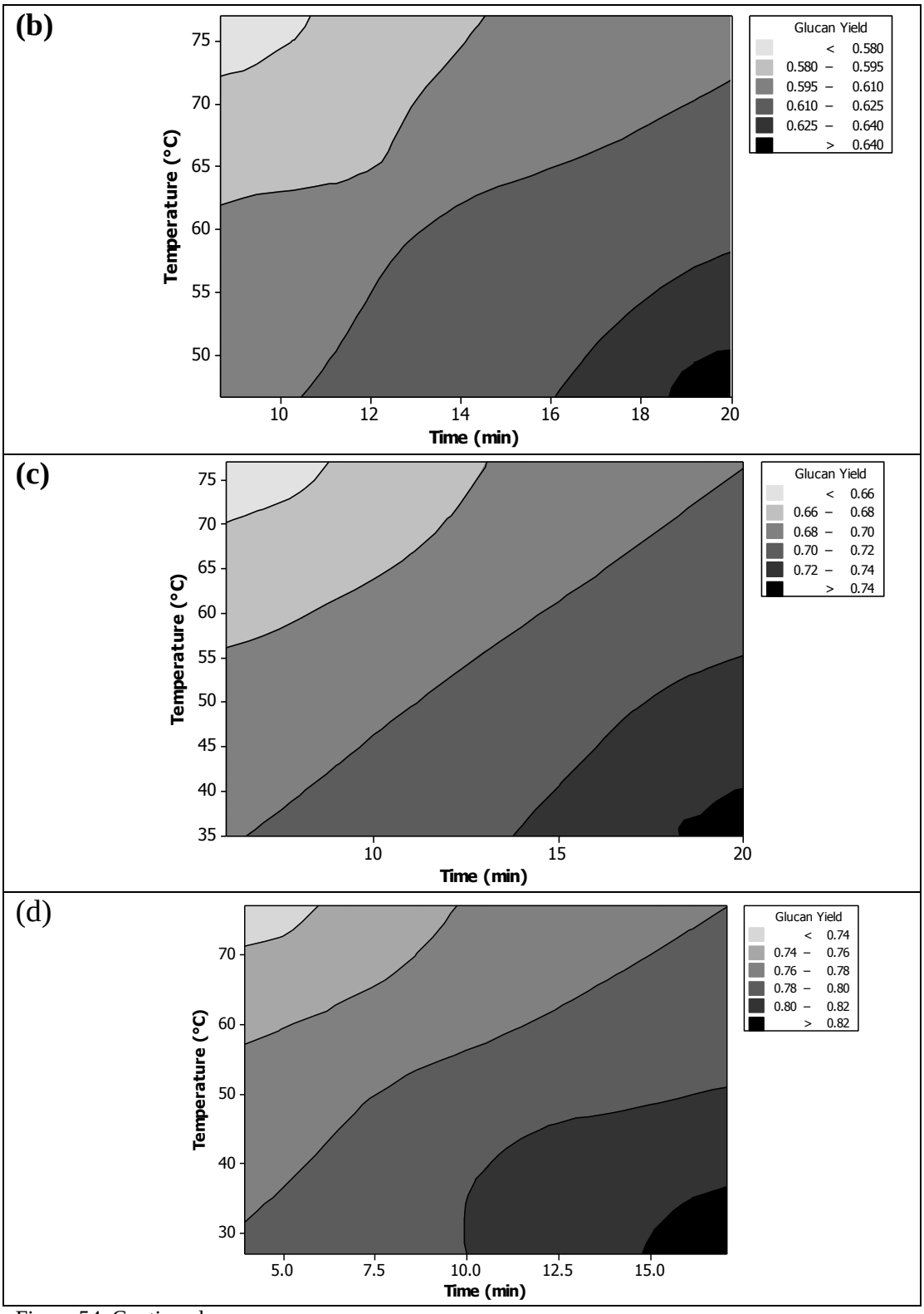

Figure 54. Continued

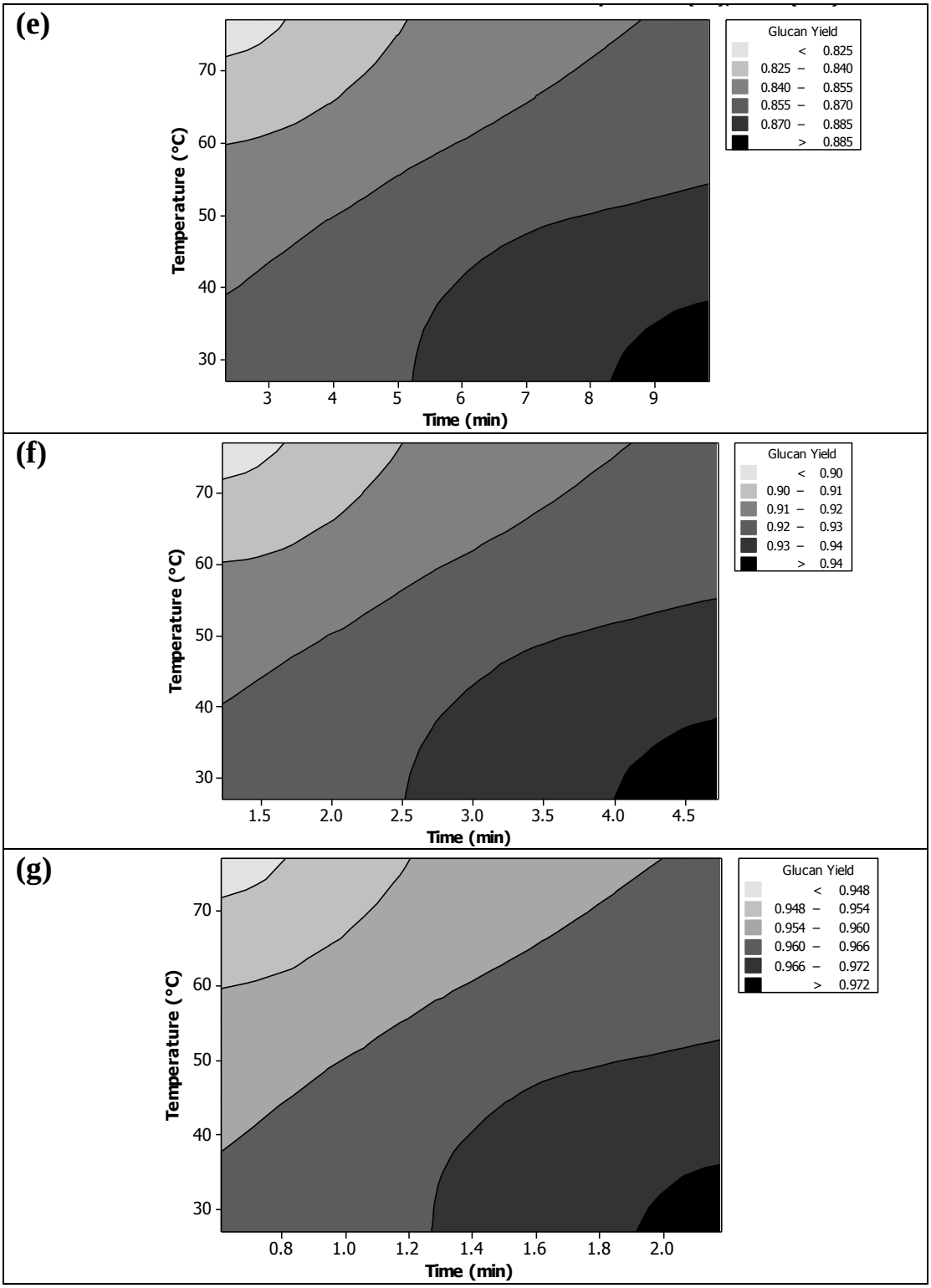

Figure 54. Continued.

Table 32. Descriptive statistics for optimized yields and selectivities in CP mode

Y_L[(a)]	Y_G[(b)]	Y_X[(c)]	S_{dG}[(d)]	S_{dX}[(e)]	Temperature (°C)	Pressure (bar)	Time (min)
0.200	0.794	0.607	1.50	1.570	131	30	600
0.300	0.880	.735	2.56	2.14	119	30	600
0.400	0.917	0.807	4.25	2.35	112	30	600
0.500	0.939	0.853	5.78	2.79	107	30	600
0.600	0.935	0.783	2.87	1.02	138	4.3	300
0.700	0.950	0.826	4.77	1.30	138	2.0	300
0.800	0.960	0.864	4.41	1.33	137	0.89	300

[(a)]g lignin, glucan or xylan remaining/g lignin, glucan or xylan in raw biomass [(b)]g lignin, glucan or xylan remaining/g lignin, glucan or xylan in raw biomass[(c)] g lignin, glucan or xylan remaining/g lignin, glucan or xylan in raw biomass [(d)] g lignin removed/g glucan or xylan removed [(e)] g lignin removed/g glucan or xylan removed

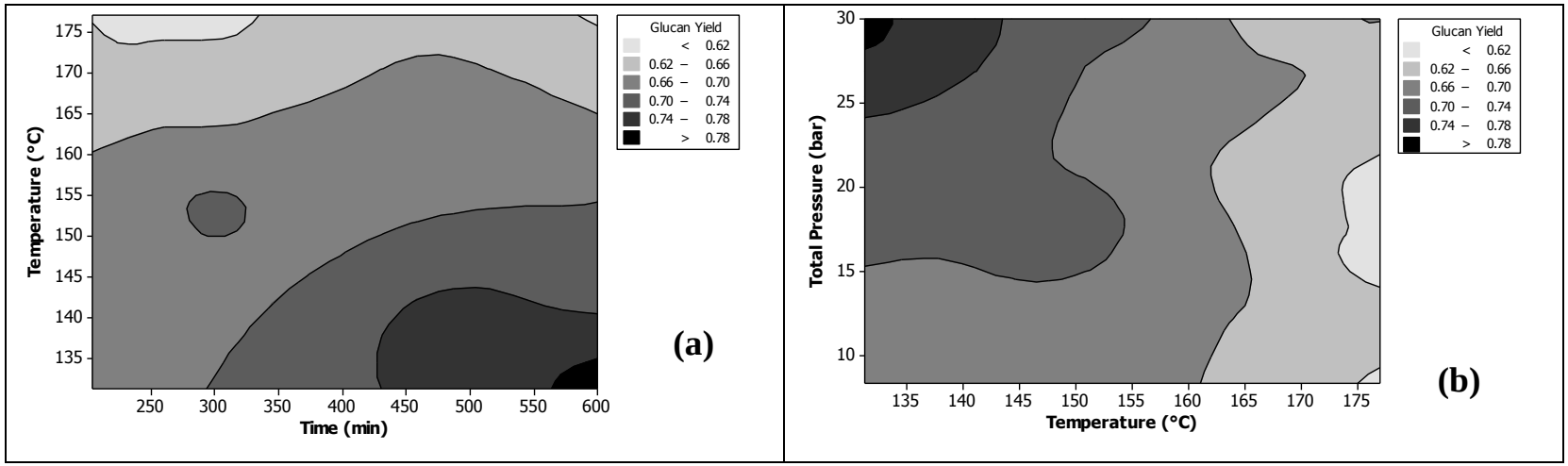

Figure 55. CP mode. Contour plots for glucan yield (g glucan/g glucan in raw biomass). Target lignin (a & b) Y_L = 0.2 (c & d) Y_L = 0.3 (e & f) Y_L = 0.4 (g & h) Y_L = 0.5 (i & j) Y_L = 0.6 (k & l) Y_L = 0.7 (m & n) Y_L = 0.8 g lignin per g lignin in raw biomass.

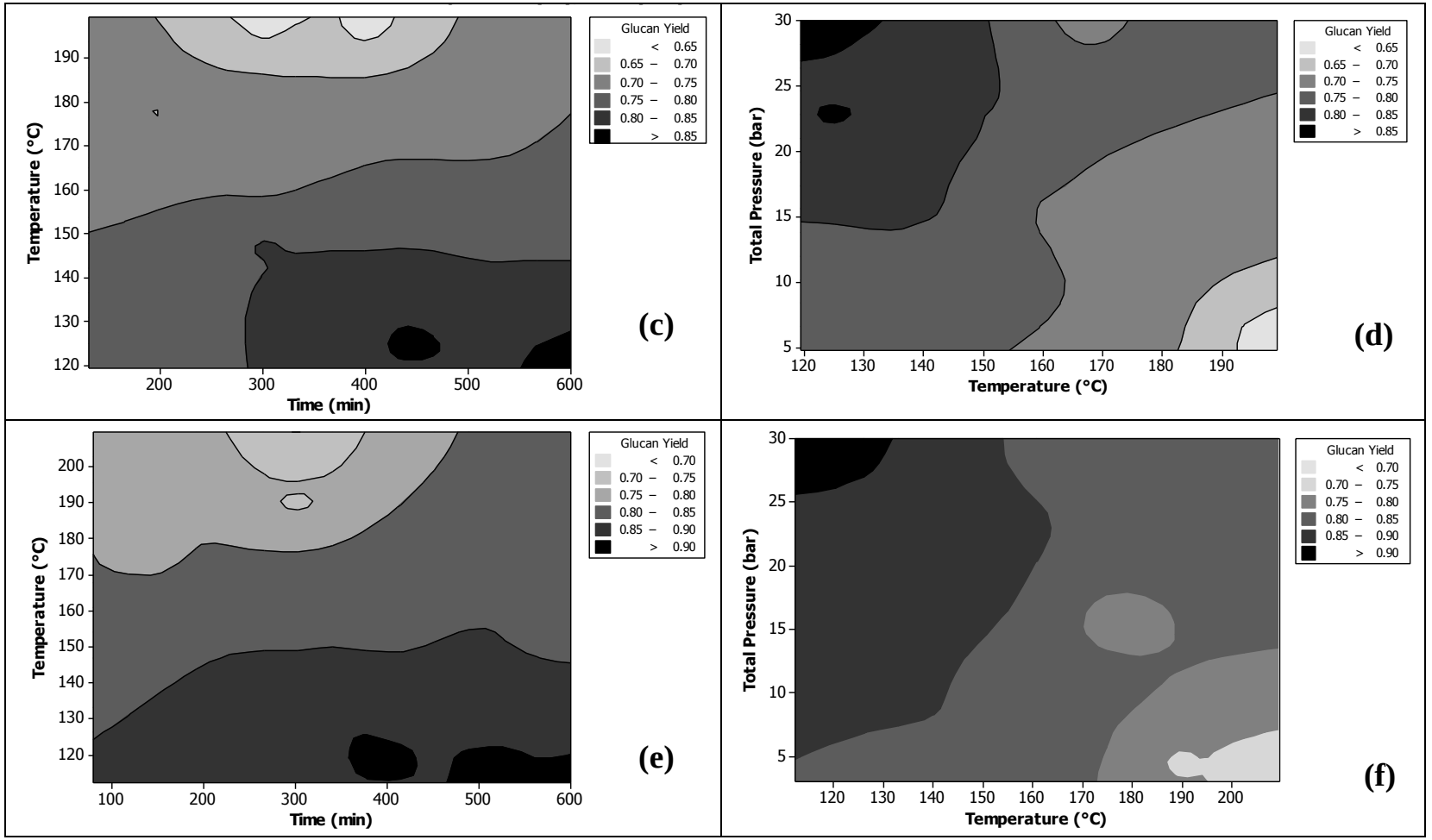

Figure 55. Continued

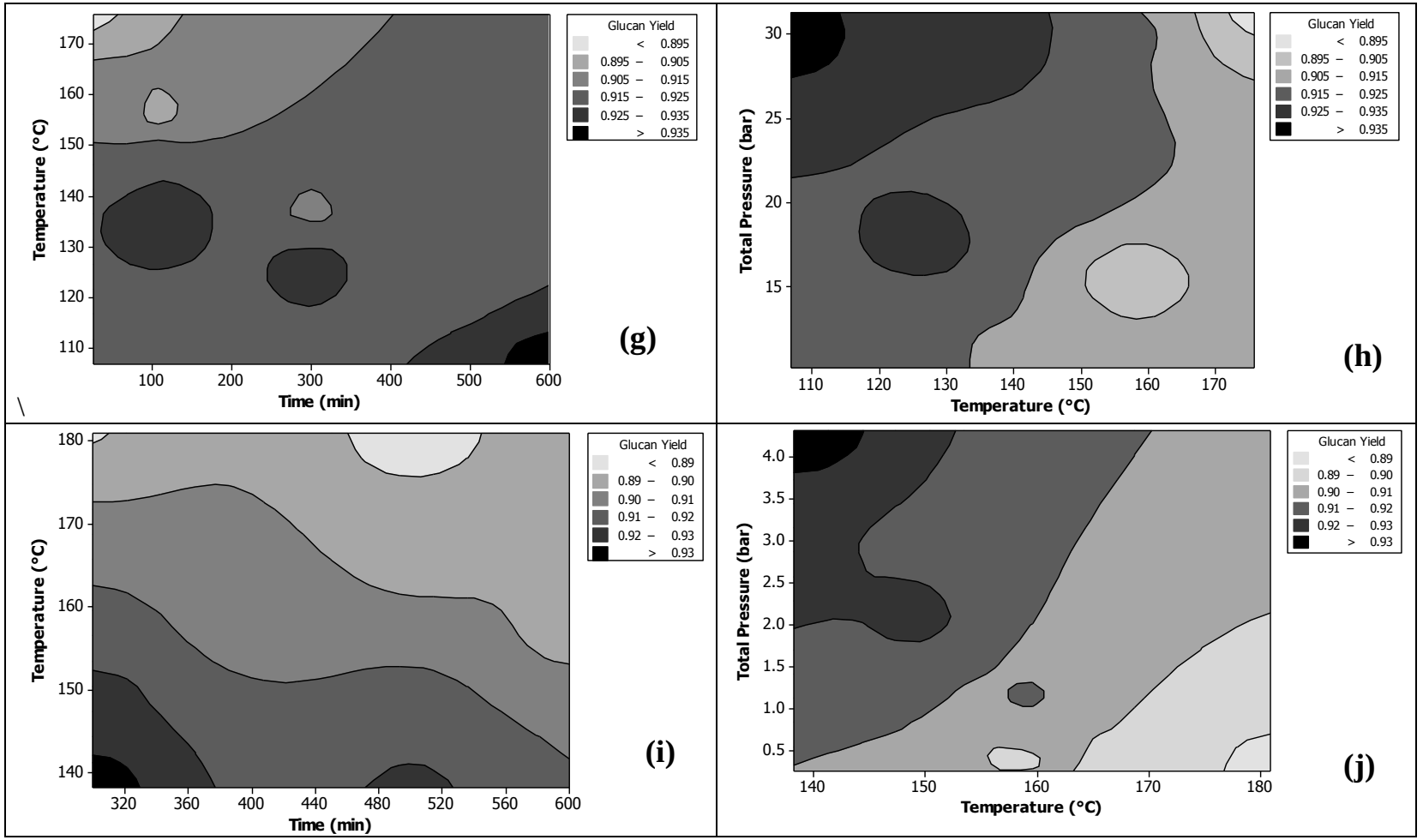

Figure 55. Continued

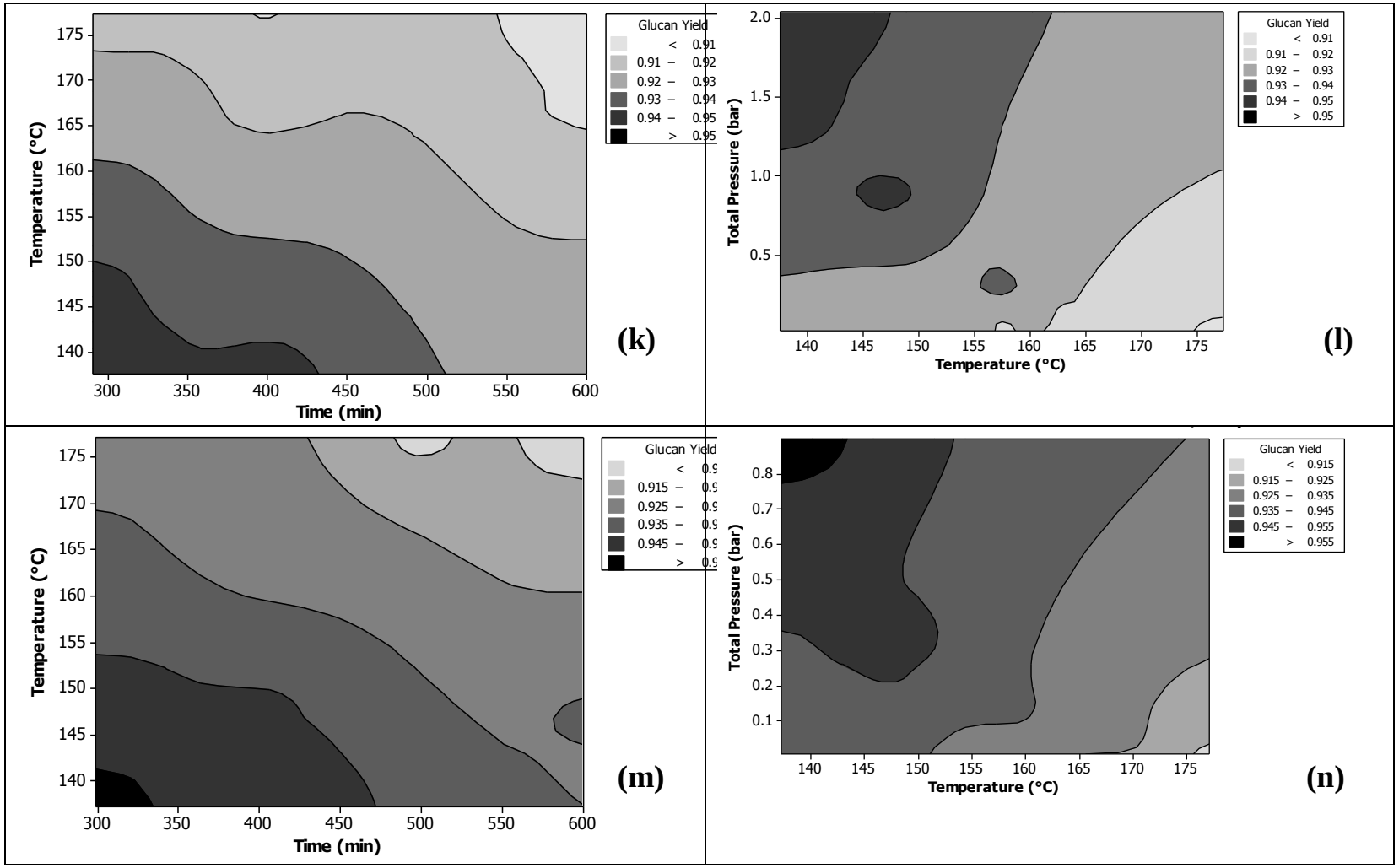

Figure 55. Continued.

Short-term varying pressure. (Table 33 and Figure 56). As in CP mode, all Y_L targets were achievable showing that VP is also very flexible and adaptable. Compared to CP, the highest Y_G for lignin targets $Y_L \leq 0.49$ g lignin/g lignin in raw biomass, were obtained using similar to longer pretreatments, similar to higher temperatures, and considerably higher pressures. For $Y_L \geq 0.50$ g lignin/g lignin in raw biomass, the best Y_G were obtained with more time, less temperature, and higher pressure; thus, in general CP requires milder conditions than VP for each Y_L target. Nevertheless, contour plots for VP show much wider ranges of pretreatment conditions that optimize Y_G compared to CP; thus, VP is more robust than CP.

In VP mode, all glucan yields were much higher than in any other mode obtaining maximum Y_G very close to 1.0 g glucan/g glucan in raw biomass for target Y_L ≥0.5 g lignin/g lignin in raw biomass. This pretreatment is also much more selective than long-term pretreatments and consequently is the recommended lime pretreatment mode for poplar wood.

Conclusions

Among all pretreatment modes included in this study (LTN, LTO, CP, and VP) VP pretreatment was the most robust and resulted in the highest Y_G for all target Y_L. Consequently, this pretreatment is recommended for poplar wood. If a reactor designed to withstand the temperatures and pressures required for VP is unavailable, LTO is to be chosen, requiring significantly higher times and at the cost of lower Y_G.

Table 33. Descriptive statistics for optimized yields and selectivities in VP mode

Y_L[(a)]	Y_G[(b)]	Y_X[(c)]	S_{dG}[(d)]	S_{dX}[(e)]	Temperature (°C)	Pressure (bar)	Time (min)
0.200	0.874	0.769	72.8	1.50	110	60	1000
0.300	0.874	0.817	98.3	2.06	114	60	600
0.400	0.964	0.844	78.2	2.44	157	6.44	715
0.500	0.992	0.869	27.2	2.36	136	8.43	600
0.600	0.996	0.878	54.4	2.37	137	5.17	500
0.700	1.000	0.963	466	3.83	107	4.34	466
0.800	1.000	0.963	728	4.73	107	1.60	1000

[(a)]g lignin, glucan or xylan remaining/g lignin, glucan or xylan in raw biomass, [(b)]g lignin, glucan or xylan remaining/g lignin, glucan, or xylan in raw biomass, [(c)] g lignin, glucan or xylan remaining/g lignin, glucan or xylan in raw biomass, [(d)] g lignin removed/g glucan or xylan removed, [(e)] g lignin removed/g glucan or xylan removed

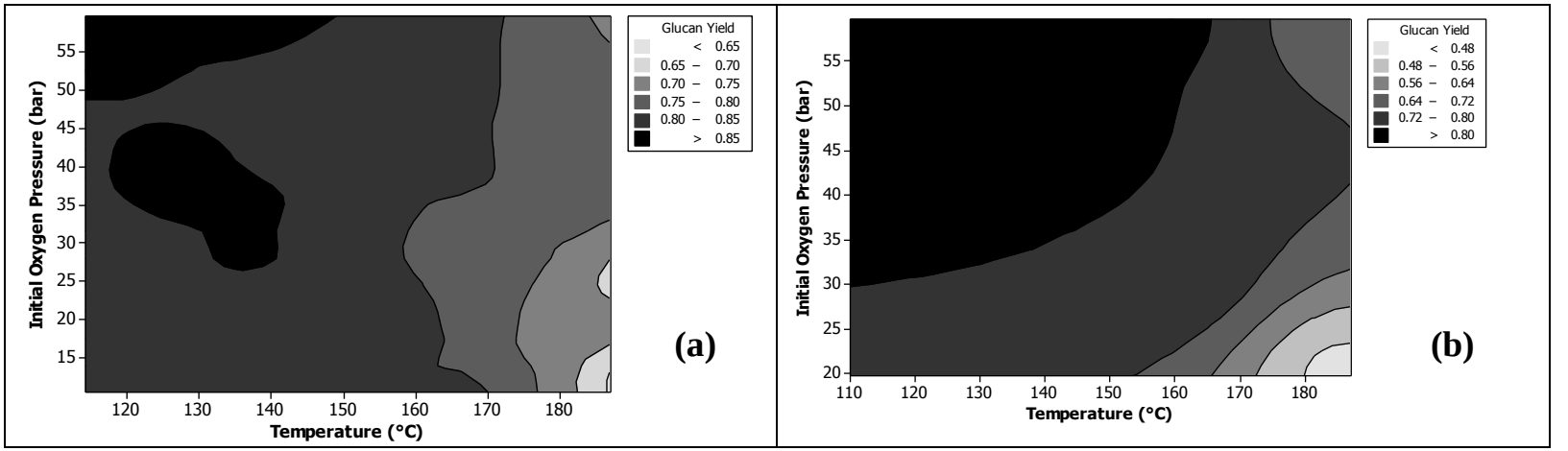

Figure 56. VP mode. Contour plots for glucan yield (g glucan/g glucan in raw biomass). Target lignin (a & b) Y_L = 0.2 (c & d) Y_L = 0.3 (e & f) Y_L = 0.4 (g & h) Y_L = 0.5 (i & j) Y_L = 0.6 (k & l) Y_L = 0.7 (m & n) Y_L = 0.8 g lignin per g lignin in raw biomass.

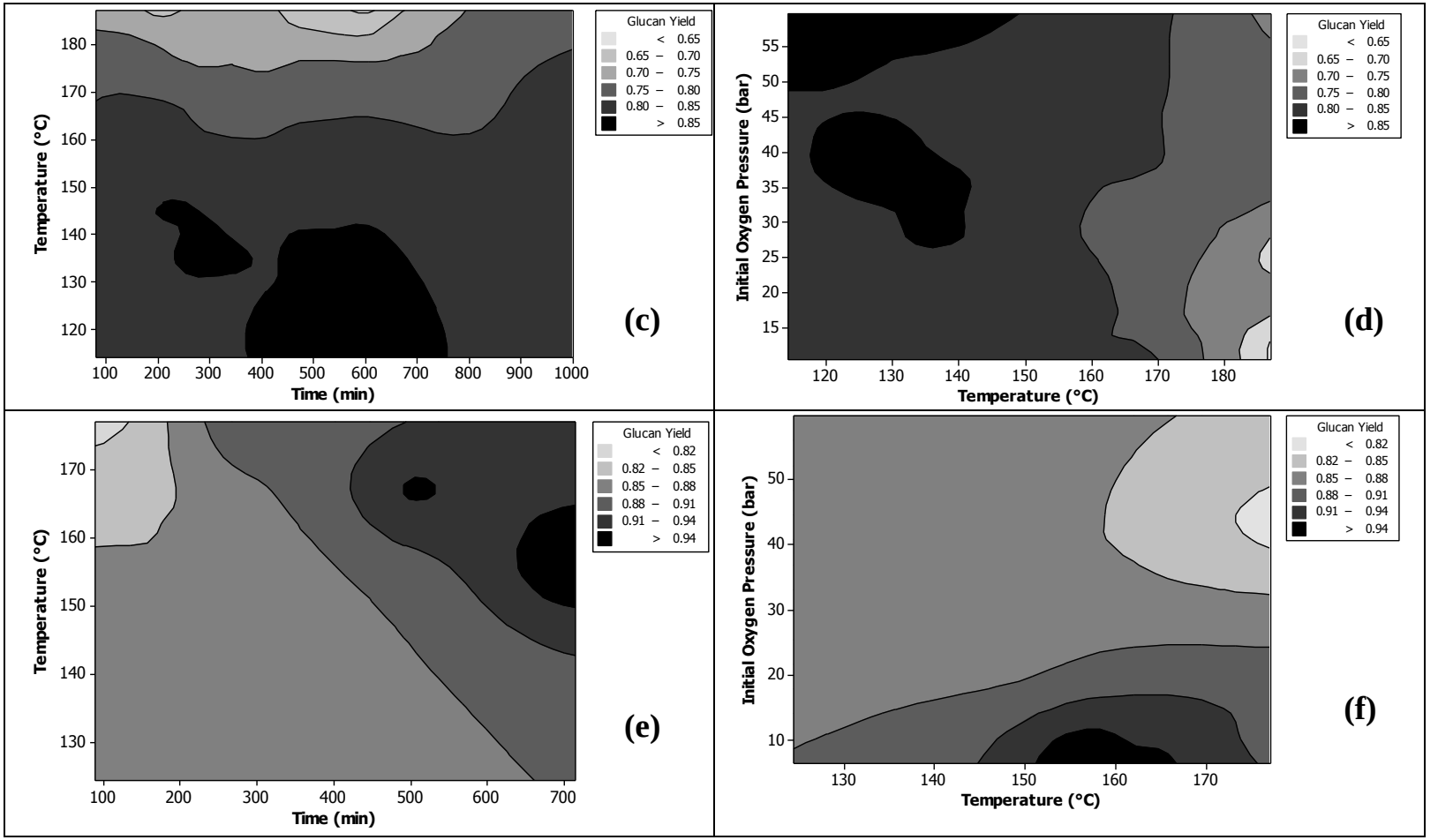

Figure 56. Continued.

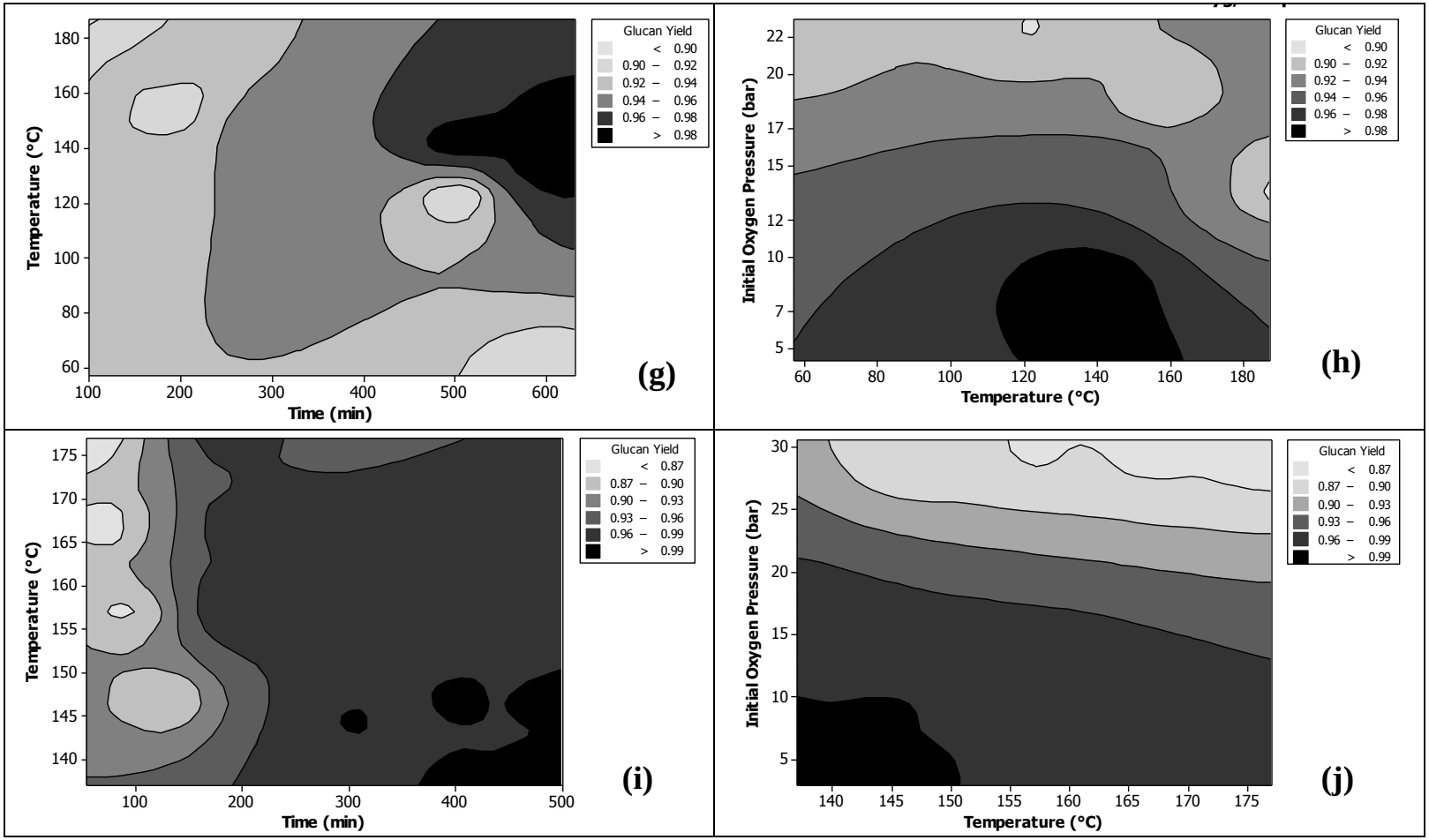

Figure 56. Continued.

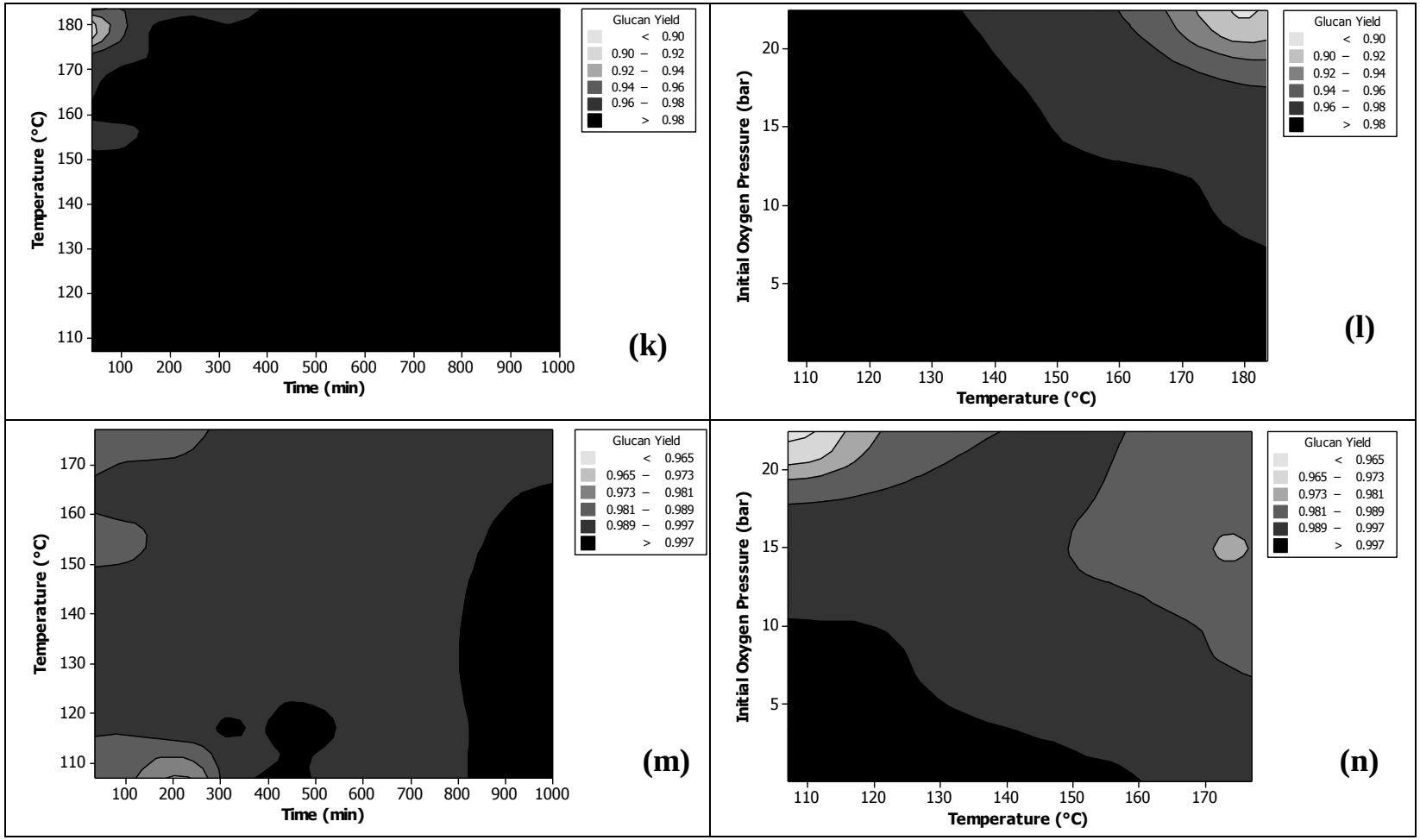

Figure 56. Continued.

CONCLUSIONS AND RECOMMENDATIONS

The study of poplar wood as feedstock for pretreatment is interesting not only because of its enormous potential and high yields but also because poplar wood has higher lignin content than most other lignocellulosic resources and therefore is more recalcitrant and challenging to pretreatment.

Lime pretreatment can use a wide range of conditions. For the purposes of this study, it was divided into four modes: long-term oxidative, long-term non-oxidative, short-term constant pressure, and short-term varying pressure. Both long-term oxidative and non-oxidative pretreatments use temperatures up to 75°C, with or without bubbling air and last up to 12 weeks. Both short-term CP and VP use temperatures up to 180°C, pressurized oxygen at constant or varying pressure and last up to 10 h.

Pretreatment was assessed for digestibility through 3-d enzymatic hydrolysis using 15 FPU/g glucan in raw biomass enzyme loading. Through non-oxidative pretreatment, it was not possible to significantly increase digestibility of poplar wood with good sugar preservation. For all oxidative pretreatment modes, pretreatment conditions that resulted in enzymatic hydrolysis yields >90 g glucan/g glucan in raw biomass were identified. For long-term oxidative pretreatment, these are 65°C and 4 weeks. For short-term constant pressure, these are 140°C, 21.7 bar, and 2 h and 160°C, 14.8 bar, and 2 h. For short-term varying pressure, these are 140°C, 7.9 bar, and 6 h. Depending on pretreatment mode, these is equivalent to overall yields > 0.80 g glucan/g

raw biomass. This result shows lime pretreatment effective on hard-to-pretreat biomass such as for poplar wood.

During oxidative lime pretreatment, oxygen is converted into hydroxyl radicals that effectively attack lignin and also degrade some carbohydrates. On the basis of compositional analysis of untreated and pretreated poplar wood, using full factorial experimental designs, it was possible to develop kinetic models for lignin and carbohydrates degradation for all pretreatment modes. These models were used to assess each pretreatment on the basis of selectivity defined in two ways: integral and differential. Short-term varying pressure pretreatment was the most selective, followed by short-term constant pressure, long-term non-oxidative and long-term oxidative.

Additionally, kinetic models were used to determine pretreatment conditions in all pretreatment modes to obtain target lignin content with maximum glucan preservation. The most robust and selective pretreatment was short-term varying pressure, which for target lignin contents >0.5 g lignin/g lignin in raw biomass resulted in theoretical glucan preservation, followed by short-term constant pressure, and long-term oxidative. Long-term non-oxidative pretreatment gave the lowest glucan preservation for all lignin targets.

On the basis of these results, short-term varying pressure pretreatment is identified as the best and recommended for poplar wood. If reactors that withstand the conditions required for varying pressure pretreatment are unavailable, long-term oxidative pretreatment is recommended.

Future studies include:

1. Systematic and parallel assessment of lime pretreatment on other types of biomass.
2. The use of magnesium or other salts to determine if they help improve lime pretreatment selectivity.
3. Include information of non-oxidative lime pretreatment at the conditions of short-term pretreatment.

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APPENDIX A

SAMPLING

This procedure is based on the NREL standard procedure "Preparation of Samples for Compositional Analysis." The purpose is to obtain a uniform and representative selection of samples for testing from a larger batch. This is done by air drying, reducing particle size, and properly mixing the biomass.

Dry

1. Spread the biomass material in a long rectangular stainless steel pan (~12×20 inch).
2. Allow air-drying (conditioning air) in a hood (controlled air velocity 100 ft/min). Do not pile the material deeper than 5 cm.
3. Turn the material at least once per day to ensure even drying.
4. After at least 4 days of drying, measure the solids content of the biomass sample following NREL "LAP Determination of Total Solids in Biomass" (Appendix B). Look for a moisture content that is less than 10%.

Mill

1. Feed the air-dried biomass into the mill. The type of mill may vary between coffee grinders for very small samples (a few grams) to lab-scale knife mills if having a total amount of biomass of 1 kg or more (e.g., Wiley mill).
2. Let the mill cool down between batches because the heat generated in the process may damage the sample and/or the mill.

Sieve

1. Stack U.S. standard brass or stainless-steel test sieve receivers in the following order (starting from the bottom): Sieve designation No. 80 (0.18 mm) and No. 20 (0.85 mm).
2. Place the milled biomass in sieve No. 20. The sample should be no more than 7 cm deep. The milled sample may be sieved in batches if necessary.
3. Place the cover on the sieve stack and secure the stack in the sieve shaker. Shake the sieves for 15 ± 1 min.
4. The fraction retained on the 20-mesh sieve (+20 mesh fraction) should be milled and sieved again or stored separately to weigh. The fraction retained on the 30 to 80+mesh sieve (− 20/+80 mesh fraction) should be retained for compositional analysis. The material in the bottom pan is the fines (− 80 mesh) fraction. Retain this material for ash analysis. It is not used in any other pretreatment or analytical procedure.

Weigh and record

1. If a particle size distribution is desired, weigh all mesh fractions to the nearest 0.1 g.
2. Determine the moisture content taking small samples of each fraction and using NREL Standard Procedure “Determination of Total Solids in Biomass” (2004) (Appendix B).
3. Report weight fractions on dry basis.

Calculate

Use the following equation to determine the weight fractions (example for the fraction that passes the 20 mesh):

$$\text{Fraction}_{20/80}\% = \left(\frac{WC_{20/80}}{WC_{20/80} + WC_{80}} \right) \times 100$$

where $WC_{20/80}$ represents the weight fraction (corrected by moisture content) that passes sieve No. 20 but does not pass sieve No. 80.

APPENDIX B

DETERMINATION OF MOISTURE CONTENT IN BIOMASS

This procedure is based on the NREL standard procedure "Determination of Total Solids and Moisture in Biomass." The purpose is to quantify the water (evaporated at 105ºC) contained in biomass material on a gravimetric basis.

Weigh

1. Accurately weigh a pre-dried aluminum foil (at 105°C) weighing dish to the nearest 0.1 mg and record this weight (W_1).
2. Thoroughly mix the sample and then weigh 1 to 5 grams (±0.1 mg) into the weighing dish.
3. Record the weight of the sample plus the weighing dish (W_2).

Dry

1. Place the sample into a convection oven at 105°C (±3)°C and dry to constant weight (±0.1% change in the amount of moisture present upon 1 h of reheating). It is advisable to dry at least 24 h.
2. Remove the sample from the oven and place in a desiccator; cool to room temperature. Weigh the dish containing the oven-dried sample to the nearest 0.1 mg and record this weight (W_3). All the samples must be run in replicate (duplicates, at minimum).
3. Repeat steps 1 and 2, reducing the drying time to ~2 h if desired, until you observe a change of weight ≤ 1%

Calculate

The meaning of the symbols W_1, W_2 and W_3 is explained in the text under "Procedure." *TS* stands for % Total Solids.

$$TS = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Conversely, the % moisture content (*MC*) of the sample is calculated as:

$$MC = 100 - TS$$

APPENDIX C

EXTRACTIVES IN BIOMASS

This procedure is based on the NREL standard procedure "Determination of extractives in biomass." It covers the determination of non-structural materials soluble in water and/or ethanol. Because extractives may interfere with the accurate determination of other components, only *extractives-free* samples (i.e., samples that have pass through this procedure) should be used to measure structural carbohydrates and lignin. Extractives percentages are used to convert compositions from an *extractives-free* basis to *as-received* basis.

Preparation

1. Determine the moisture content of the sample (Appendix B).
2. Dry a boiling flasks (500-mL capacity) in a 105(±5)°C drying oven for a minimum of 15 hours.
3. After cooling in a desiccator, record its oven-dry weight (ODW) to the nearest 0.1 mg.
4. Add weighed boiling stones (beads) to the boiling flask.
5. Add about 250 mL of 190-proof ethyl alcohol (water if water extractives are to be determined) to the flask.
6. Add 2–8 g of sample to a cellulose extraction thimble (single thickness, Whatman®) and record the weight to the nearest 0.1 mg.
7. Insert the thimble into the Soxhlet tube.

8. Assemble the Soxhlet apparatus (from bottom to top: heating mantel, boiling flask, Soxhlet tube and condenser).

Solvent extraction procedure

1. Adjust the heating mantles to provide a minimum of 6–10 siphon cycles per hour and reflux for 16− 24 hours.
2. When reflux time is complete, turn off the heating mantles and allow the glassware to cool to room temperature.
3. Remove the thimble and transfer the extracted solids, as quantitatively as possible, onto cellulose filter paper in a Buchner funnel.
4. Wash the solids with approximately 50 mL of fresh 190-proof ethanol (water if that was the solvent).
5. Allow the solids to dry using vacuum filtration or air dry.
6. Combine any solvent from the Soxhlet tube with the solvent obtained after vacuum filtration and the solvent still contained in the boiling flask.
7. Use a rotary evaporator equipped with a water bath set to 40 (± 5)°C and a vacuum source. The vacuum source should be sufficient to remove solvent without extreme bumping. Continue to remove solvent until all visible solvent is gone. Place the flask in a vacuum oven at 40 (±2)°C for 24 hours. Cool to room temperature in a desiccator. Weigh the flask or tube and record the weight to the nearest 0.1 mg.

Calculate

Use the following equation to obtain the extractives content:

$$\% \text{ Extractives} = \frac{WFR - WF}{ODW} \times 100$$

where

WFR = Weight of the flask plus residue

WF = Weight of the flask

ODW = Weight of the sample corrected by its moisture content (or dry weight)

APPENDIX D

DETERMINATION OF CARBOHYDRATES, LIGNIN AND ACETYL CONTENT IN BIOMASS

This procedure is based on the NREL standard procedure "Determination of Structural Carbohydrates and Lignin in Biomass (2004)". The purpose is quantify the following components of biomass: cellobiose, glucose, xylose, galactose, arabinose, mannose, lignin (insoluble lignin and soluble lignin), and acetic acid.

Prepare sample

1. Determine the moisture content of the sample according to NREL Standard Procedure "Determination of Total Solids and Moisture in Biomass" (Appendix B). The moisture content must be 10% or less.
2. Grind if necessary. The particle size must be in the range –20/+80 mesh. Deviation to a larger or smaller particle size may result in bias in both the lignin and the carbohydrates content.
3. Have the sample extractives free, running the procedure "Extractives in biomass" explained in Appendix C before this procedure.

Prepare crucibles

Filtering crucibles (25-mL, porcelain, medium porosity, Coors #60531 or equivalent) are necessary in this procedure. An appropriate number of filtering crucibles must have been prepared at least one day before running this procedure. The correct crucibles

preparation and permanent supervision of the analytical balance accuracy are fundamental to obtain an accurate, consistent result.

1. The preparation of the crucibles starts by ignition of the crucibles in a muffle furnace at 575 (±25) °C for a minimum of 4 h.
2. After ignition, the crucibles must be removed from the furnace directly into a desiccator.
3. Let them cool for exactly 1 h and weigh them to the nearest 0.1 mg and record this weight.
4. Place them back in the furnace and ash to constant weight defined as less than ± 0.3 mg change in the weight upon 1 h of reheating.

Prepare calibration curve

It is a series of sugar solutions of known concentration used to calculate an unknown sample sugar concentration. Prepare them either in advance or after running this procedure. It is highly recommended to have to one calibration curve ready for each HPLC run. The range of the concentrations is suggested as 0.1, 0.5, 1, 2, 3, 4, 5 mg/mL for D-cellobiose, D-(+)glucose, D-(+)xylose, and D-(+)mannose.

Concentrated acid hydrolysis

1. Weigh 0.3 (± 0.01) g of the sample and place it into a labeled 16×100 mm test tube and record the weight to the nearest 0.1 mg. Run the NREL Standard Procedure "Determination of Total Solids in Biomass" (Appendix B) at the same time, to accurately measure the percent solids for correction.

2. Add 3.00 (±0.01 mL) of 72% sulfuric acid to each pressure tube. Place the pressure tube in a water bath set at 30 (±3)°C and incubate the sample for 60 (± 5) minutes.
3. Using a Teflon stir rod, stir the sample every 5 to 10 min without removing the sample from the bath.

Prepare Sugar Recovery Standards (SRS)

This set of sugars that is used to correct for losses due to sugar degradation during dilute acid hydrolysis. For poplar wood, SRS should include D-(+)glucose, D-(+)xylose, and D-(+)mannose. SRS sugar concentrations should be chosen to most closely resemble the concentrations of sugars in the test sample (i.e., for a sample with 43% of glucan, 15% of xylan and 3% of mannose, it is necessary to weigh about 0.130 g glucose, 0.045 g xylose and 0.009 g mannose). The SRS may be prepared during the concentrated acid hydrolysis step. This analysis has two purposes: check the HPLC calibration and avoid errors such as balance calibration when comparing SRS concentration before and after dilute hydrolysis.

1. Weigh the required amount of sugar (to the nearest 0.1 mg), transfer it to a pressure glass bottle, add 84.0 mL deionized water and 3 mL of 72% sulfuric acid.
2. Immediately shake vigorously and transfer a 20-mL aliquot into a 50-mL Erlenmeyer flask and neutralize this sample as explained below in the section "neutralization." This will allow the analysis on HPLC of the initial sugar concentration of the SRS.

Dilute acid hydrolysis

1. Once the time for the concentrated acid hydrolysis has elapsed, remove the tubes from the water bath.

2. Dilute the acid to a 4% concentration by adding 84.00 (±0.04) mL deionized water with an automatic burette.
3. Seal the bottles and place them in an autoclave.
4. Autoclave samples and sugar recovery standards for 1 h at 121°C.
5. Allow the hydrolyzates to slowly cool to room temperature before removing the caps.

Acid insoluble lignin analysis

1. Vacuum filter the autoclaved hydrolysis solution through one of the prepared filtering crucibles.
2. Capture the filtrate in a filtering flask.
3. Transfer an aliquot (10 to 50 mL) into a sample storage bottle. This sample will be used to determine acid-soluble lignin as well as carbohydrates and acetyl content.
4. Using a minimum of 50 mL of hot deionized water to quantitatively transfer all remaining solids out of the pressure bottle into the filtering crucible.
5. Dry the crucible and acid insoluble residue at 105 (±3) °C until a constant weight is achieved, minimum overnight, better 24 hours or more.
6. Remove the samples from the oven and cool in a desiccator.
7. As accurately as possible, record the weight of the crucible and dry the residue to the nearest 0.1 mg.
8. Place the crucibles and residue in the muffle furnace at 575 (±25) °C for 24 (±6) hours.

9. Carefully remove the crucible from the furnace directly into a desiccator and cool for exactly 1 h.
10. Weigh the crucibles and ash to the nearest 0.1 mg and record the weight. Place the crucibles back in the furnace and ash to a constant weight.

Acid soluble lignin analysis

This analysis must be performed within 6 h of hydrolysis on a UV-Visible spectrophotometer (background, deionized water) using the hydrolysis liquor aliquot obtained after vacuum filter the autoclaved hydrolysis solution.

1. Measure the absorbance of the sample at 320 nm on a UV-Visible spectrophotometer.
2. Using deionized water dilute the sample as necessary (a dilution factor of 3 is recommended) to bring the absorbance into the range of 0.2–1.0, recording the dilution.
3. Record the dilution factor and the absorbance to three decimal places.

Carbohydrates analysis

1. Transfer 20 mL of the hydrolysis liquor obtained after the filtering step to a 50-mL Erlenmeyer flask.
2. Use calcium carbonate to neutralize each sample to pH 5–6. Allow the sample to settle and decant off the supernatant. The pH of the liquid after settling will be approximately 7.

3. Centrifuge the sample to eliminate the calcium carbonate, and prepare the sample for HPLC analysis by passing the decanted liquid through a 0.2-µm filter into an autosampler vial.
4. Seal and label the vial. Analyze the calibration standards, SRS before and after hydrolysis, and samples by HPLC using a Biorad Aminex HPX-87P column equipped with the appropriate guard column. HPLC conditions follow:

    Injection volume: 20 µL

    Mobile phase: HPLC grade water, 0.2-µm filtered and degassed

    Flow rate: 0.55 mL/min

    Column temperature: 85°C

    Detector temperature: room temperature

    Detector: refractive index

    Run time: 20 minutes

If cellobiose and oligomeric sugars are detected in levels greater than 3 mg/mL, incomplete hydrolysis occurred and fresh samples should be hydrolyzed and analyzed. Peaks before cellobiose may indicate high levels of sugar degradations products in the previous sample, which indicates over hydrolysis. All samples from batches showing evidence of over-hydrolysis should have fresh samples hydrolyzed and analyzed.

Acetyl content

1. Prepare 0.01-N sulfuric acid for use as a HPLC mobile phase. (278-µl concentrated sulfuric acid in a 1-L volumetric flask, bringing to volume with HPLC-grade water).

2. Filter this mobile phase through a 0.2-µm filter and degas before use. Prepare a series of calibration standards containing acetic acid in a range of 0.005 to 0.5 mg/mL.
3. Prepare the sample for HPLC analysis by passing a small aliquot of the liquor through a 0.2-µm filter into an autosampler vial.
4. Seal and label the vial. Analyze the calibration standards, CVS, and samples by HPLC using a Biorad Aminex HPX-87H column equipped with the appropriate guard column. HPLC conditions follow:

    Sample volume: 50 µL

    Mobile phase: 0.01-N sulfuric acid, 0.2-µm filtered and degassed

    Flow rate: 0.55 mL/min

    Column temperature: 65°C

    Detector temperature: room temperature

    Detector: refractive index

    Run time: 45 minutes

Calculate

Acid-insoluble lignin:

$$\% AIL = \frac{(WCR - WC) + (WCA - WC)}{ODW} \times 100$$

where

%*AIL* = Percentage of acid insoluble lignin

WCR = Weight of crucible plus residue

WC = Weight of crucible

WCA = Weight of crucible plus ash

ODW = Dry weight of the sample (or weight corrected by moisture content)

Acid-soluble lignin

$$\% \, ASL = \frac{UV \cdot 87 \cdot DF}{11.4 \cdot ODW} \times 100$$

where:

%ASL = Percentage of acid insoluble lignin

UV = Average UV-Vis absorbance of the sample at 320 nm

DF = Dilution factor

ODW = Dry weight of the sample (or weight corrected by moisture content)

The values 87 and 11.4 stand for volume of the filtrate and absorptivity of poplar wood at 320 nm, respectively

$$\% \text{ Total Lignin} = \% \, AIL + \% \, ASL$$

Percentage of recovery of SRS

$$PR = \frac{SRS_A}{SRS_B}$$

where:

PR = percentage of recovery of SRS

SRS_A = Concentration of sugar as measure by HPLC before dilute acid hydrolysis

SRS_B = Concentration of sugar as measure by HPLC after dilute acid hydrolysis

Concentration of carbohydrates:

$$C_i = \frac{C_{HPLC} \cdot AC \cdot 87}{PR \cdot ODW \cdot 10}$$

where:

C_i = Concentration of Sugar *i*

C_{HPLC} = Concentration of Sugar *i* as given by HPLC

PR = Percentage of recovery of SRS

AC = Anhydro correction to calculate the concentration of polymeric sugars from the corresponding concentration of monomeric sugars. It is 0.88 for glucose and mannose and 0.9 for xylose.

ODW = Dry weight of the sample (or weight corrected by moisture content)

The values 87 and 10 stand for volume of the sample and conversion units factor, respectively.

Acetate content

$$\% ACE = \frac{C_{AHPLC} \cdot 87 \cdot 0.683}{ODW} \times 100$$

C_{AHPLC} = Concentration of acetic acid as given by HPLC

ODW = Dry weight of the sample (or weight corrected by moisture content)

The values 87 and 0.683 stand for volume of the sample and conversion factor from acetic acid to acetate, respectively.

APPENDIX E

DETERMINATION OF ASH IN BIOMASS

This procedure is based on the NREL standard procedure "Determination of Ash Biomass." The purpose is to measure the amount of inorganic material in biomass, either structural or extractable, as part of the total composition.

Prepare materials and samples

1. Determine the moisture content of the samples using the NREL Standard Procedure "Determination of Total Solids and Moisture in Biomass" (Appendix B) at the time when the sample is weighed.
2. Label the appropriate number of crucibles (ashing crucibles, 50-mL, porcelain) with a porcelain marker and place them in the muffle furnace at 575 (±25) °C for a minimum of 4 h.
3. Remove the crucibles from the furnace directly into a desiccator. Cool for exactly 1 h.
4. Weigh the crucibles to the nearest 0.1 mg and record this weight.
5. Place the crucibles back into the muffle furnace at 575 (± 25)^{o}C and dry to constant weight.

Ignite and ash

1. Weigh 0.5 to 2.0 g, to the nearest 0.1 mg, of the sample into the tared crucible. Record the sample weight.

2. Using a burner and clay triangle with stand, place the crucible over the flame and let the sample burn until no more smoke or flame appears.
3. Place the crucibles in the muffle furnace at 575 (± 25) ^{o}C for 24 (± 6) h.
4. When handling the crucible, protect the sample from drafts to avoid mechanical loss of sample.
5. Carefully remove the crucible from the furnace directly into a desiccator and cool for exactly 1 h.
6. Weigh the crucibles and ash to the nearest 0.1 mg and record the weight. At 575 (± 25) °C ash to constant weight.

Calculate

$$\% \text{ Ash} = \frac{WCA - WC}{ODW} \times 100$$

where

%Ash = Percentage of ash

WCA = Weight of the crucible plus ash

WC = Weight of the crucible

ODW = Dry weight of the sample (corrected by moisture)

APPENDIX F

STARTING UP PROCEDURE FOR THE SHORT-TERM PRETREATMENT REACTOR SYSTEM

The purpose of this procedure is to bring the short-term pretreatment reactor system to operating conditions. The steps are summarized as follows:

1. Tightly close 5-inch (0.127 m) long, 1.5-inch (0.0381 m) inside diameter 304 stainless steel nipples on one end using a stainless steel cap and Teflon tape. This will become one reactor for pretreatment.
2. Mix well 8 g of biomass, 120 mL of water, and 4 g of lime inside this reactor.
3. Tightly close the other end of the reactor using another 304 stainless steel cap.
4. If VP mode, load oxygen into the reactor to the desired initial pressure.
5. Attach the reactor to a holder, put the holder inside an oven preheated to the pretreatment temperature, and wait about 40 min for the reactor to equilibrate temperature.
6. Start shaking mechanism (either rotator or swing arm)
7. If CP mode, open the oxygen line to load oxygen in the reactors to the desired total pressure.
8. When pretreatment time has elapsed, close oxygen valves (if CP mode), stop shaking, turn off the oven, and fully open it to allow for cool down. If possible,

cooling down may be speeded up by putting the reactors in contact with an ice-water bath.

9. Once the temperature is low enough, carefully open the reactors allowing slow depressurizing while opening.
10. Carefully and completely transfer all the reactor contents into a centrifuge bottle for sample preparation and analysis.

APPENDIX G

STARTING UP PROCEDURE FOR THE LONG-TERM PRETREATMENT REACTOR SYSTEM

The purpose of this procedure is to bring the long-term pretreatment reactor system to operating conditions. The steps are summarized as follows:

1. Fill water into the water tank. Nearly full level is recommended.
2. Turn on the centrifugal pump to circulate water. Refill sufficient water into the tank to maintain a nearly full level.
3. Check for leaks in the system and correct them as needed.
4. Turn on the temperature controller to heat up the circulating water to the set temperature.
5. Operate the whole system to reach a steady state. Steps 1 through 5 can be omitted in the case of pretreatment at 25ºC.
6. Transfer a mixture of 15.0 g dry weight of the raw biomass and 7.5 g of calcium hydroxide and 110 mL of water to the reactors using a funnel. Use 40 mL of distilled water to rinse the spatula and the container of the mixture and transfer all remnants to the reactor.
7. Tightly cap the reactor and connect the bubble indicator (previously filled with 20 − 25 mL of distilled) to measure the gas flow rate.
8. Slowly open the appropriate valve to supply nitrogen for non-oxidative pretreatment or air for oxidative pretreatment. Confirm bubble formation in the bubble indicator.

Adjust the gas flow rate to achieve at 2 – 3 bubbles/second using clamp placed in the inlet tube at the bottom of the reactor.

9. Regularly check gas flow rate, seals, water levels in the cylinder filled with water and in the tank, and working temperatures in all reactors.
10. After the pretreatment time has elapsed, remove the reactors and cool down to room temperature.

APPENDIX H

NEUTRALIZATION OF LIME AFTER PRETREATMENT

This procedure has a double purpose: determine the lime consumption during pretreatment and neutralize the sample to render it ready for analytical procedures that may be affected for pH.

Prepare sample

1. Once the pretreatment time is elapsed, let the reactor cool to room temperature.
2. Transfer its contents to a 1-L centrifuge bottle, using distilled water to rinse and move all the material as completely as possible.

Procedure

1. Set up titration apparatus (buret, clamp, magnetic stirrer and a well-calibrated pH meter).
2. Place a magnetic bar into the centrifuge bottle containing pretreated biomass slurry and place the bottle on the magnetic stirrer.
3. Dip the pH probe inside of the bottle to measure the pH of the slurry. Fill 5-N HCl solution in the buret and clamp it over the bottle.
4. Record the volume (V_i). Slowly drop the acid into the bottle up to the end point (pH 7.00).
5. Provide enough time (more than 1 h) to ensure the pH of the slurry is stabilized. Record the volume left in the buret (V_f).

Calculate

Use the following equation to determine the lime consumption during pretreatment:

$$W_{ca(OH)_2} = \frac{1 \quad mol \quad Ca(OH)_2}{2 \quad mol \quad HCl} \times \frac{N_{HCl} \cdot (V_i - V_f)}{1000} \times M_{Ca(OH)_2}$$

where,

$W_{Ca(OH)2}$ = The amount of lime, $Ca(OH)_2$, unreacted (g)

N_{HCl} = Normality of HCl solution

$V_i - V_f$ = Total volume of HCl solution to titrate the biomass slurry (mL)

$M_{Ca(OH)2}$ = Molecular weight of $Ca(OH)_2$, 74.092 g/mol

APPENDIX I

WASHING BIOMASS PROCEDURE AND RECOVERY YIELD OF TOTAL MASS

This procedure is run immediately after the neutralization of the sample. Its purpose is to eliminate the pretreatment liquor from the sample. The weight loss of biomass due to pretreatment (recovery yield of total mass) is also determined.

Preparef materials

1. Dry a plastic container (about 500-mL capacity) and Whatman 934/AH glass fiber filter paper (particle retention = 1.5 µm, Fisher Scientific Co., Pittsburgh PA) in a 45ºC oven for 24 h or longer.
2. Let them cool in a dessicator. Record their weights to the nearest 0.1 mg.

Wash

1. After neutralizing the sample as explained in the Appendix H, continue stirring for 15 min.
2. Centrifuge the water/poplar wood mixture at 4000 rpm for 15 min. During the centrifuge period, set up a vacuum filtration apparatus using a Buchner funnel and one of the pre-dried/pre-weighed filter papers.
3. Carefully decant the water into the Buchner funnel with vacuum filtration. Decant as much water as possible being careful not to lose much solids.
4. Fill the centrifuge bottle with 750 mL of fresh distilled water. Observe the filtrate color.

5. Stir, centrifuge, decant, and fill the centrifuge bottle with fresh distilled water as many times as necessary until the filtrate becomes clear. If it takes too long to filter, replace the old filter with one of the other previously dried-and-weighed filter papers.

Determine weight loss

1. After completing the washing, transfer all the poplar wood from the centrifuge bottle to the prepared 500-mL container.
2. Transfer all the solids as quantitatively as possible to the container using water. Dry the biomass and the filter papers at 45ºC for 24 h or longer.
3. Cool the biomass and filters in a desiccator until they reach room temperature.
4. Weigh them and record the values to the nearest 0.1 mg. After subtracting the weight of the containers and filter paper, the net weight of the poplar wood is obtained (W_2).
5. Immediately after, using about 0.3 – 0.5 g of this 45ºC-dried washed biomass, determine the moisture content as described in the NREL Standard Procedure "Determination of Total Solids and Moisture in Biomass" (Appendix B) (X_2).

$$Y = \frac{W_2 \times (1 - X_2)}{W_1 \times (1 - X_1)}$$

where

Y = Total yield, g treated bagasse/g untreated bagasse

W_1 = Weight of the washed raw biomass before pretreatment

X_1 = Moisture content of the washed and air dried raw biomass (W_1), g H_2O/g total weight

W_2 = Weight of the 45ºC-dried poplar wood in the 500-mL container and filter papers

X_2 = Moisture content of the 45ºC-dried biomass (W_2), g H_2O/g total 45ºC-dried weight.

APPENDIX J

ENZYMATIC HYDROLYSIS

This procedure is based on the NREL standard procedure "Enzymatic Saccharification of Lignocellulosic Biomass." The purpose is to determine the maximum extent of digestibility possible after the enzymatic saccharification of cellulose from untreated or pretreated lignocellulosic biomass.

Prepare sample and analysis

1. Before running this procedure, make sure the biomass has been neutralized and washed because deviations in the pH (too acidic or alkaline) affect the enzymatic hydrolysis yields greatly.
2. Determine the moisture content of the samples using the NREL Standard Procedure "Determination of Total Solids and Moisture in Biomass" (Appendix B) in advance.
3. Measure glucan content of the sample according to the method described in Appendix D prior to this analysis.
4. The recovery yield of total mass must be known beforehand (Appendix I).
5. The enzyme activity should be measured to assure good conservation during the storage (use NREL Standard Procedure "Measurement of Cellulase Activities.")
6. Calculate the amount of biomass equivalent to 0.1 g of glucan in raw biomass as follows:

$$B = \frac{0.10}{G \cdot TS}$$

where:

B = Biomass to be weighed

G = Glucan fraction in the treated biomass

TS = Solid fraction in the sample (equivalent to 1 minus moisture content)

Also calculate the amount of enzyme to be added as:

$$E_1 = \frac{\left(\frac{0.1}{Y_G}\right) \cdot E}{EA}$$

E_1 = Amount of enzyme to be added

E = Enzyme loading = 15 FPU/g glucan in raw biomass

EA = Enzyme activity

Y_G = Pretreatment yield of glucan

Enzymatic hydrolysis procedure

1. Prepare citric acid solution by dissolving 210 g of citric acid monohydrate in 1000 mL of distilled water, then adjust the pH to 4.5 by adding NaOH. This stock solution is 1 M and may be stored. Dilute to 0.1 M before using in enzymatic hydrolysis procedures.
2. Weigh B g of biomass into a labeled 20-mL glass scintillation vial.
3. Add sodium citrate buffer (5 mL, 0.1 M, pH 4.8), tetracycline (40 µL, 10 mg/mL in 70% ethanol), cycloheximide (30 µL, 10 mg/mL in distilled water) and an amount of distilled water (W), HPLC grade, equal to W=5–B–E_1–E_2 where B and E_1 were

defined before and E_2 is the required amount of cellobiase to obtain 60 CBU/g. This is to bring the volume in the vial to 10 mL (after adding enzymes).

4. Measure the pH in the vials and adjust to 4.8 with either a saturated solution of sodium hydroxide or acetic acid as necessary.
5. Close the vials and preheat them in a rotary incubator (Amerex Instruments Inc, Lafayette, CA) at a speed of 105 rpm and a temperature of 50ºC for 1 h. The vials should be held in the incubator at a minimum angle of 45º to assure good mixing.
6. Take the vial briefly out of the incubator, add the enzymes, both at a time and place the vial back in the incubator. Record the time. If more than a sample is run in a batch, it is advisable to add the enzymes at specific intervals of time between the samples, 30 s to 1 min are recommended.

Analyze

1. Once the enzymatic hydrolysis time has elapsed, take the samples out of the incubator in the same order as the enzyme was applied and with the same interval of time between the samples.
2. Put the closed vials in a boiling water bath to denature the enzymes and let them heat for 15 min. Place the vials in a mixture of ice and water and let them cool down for 10 min.
3. Transfer the vials contents to labeled 15-mL centrifuge tubes and centrifuge for 10 min at 4000 rpm to eliminate the solid residue.
4. Dilute the decanted liquid (if necessary) with distilled water (HPLC grade) recording the dilution factor.

5. Prepare the sample for HPLC analysis by passing the decanted diluted liquid through a 0.2-µm filter into an autosampler vial. Seal and label the vial.
6. Analyze calibration standards (to prepare calibration standards use guidelines in Appendix D) and samples by HPLC using a Biorad Aminex HPX-87P column equipped with the appropriate guard column. HPLC conditions follow:

   Injection volume: 20 µL, dependent on concentration and detector limits

   Mobile phase: HPLC grade water, 0.2-µm filtered and degassed

   Flow rate: 0.55 mL/min

   Column temperature: 85°C

   Detector temperature: Room temperature

   Detector: refractive index

   Run time: 35 minutes

Calculate

$$\%\ \text{digestion} = \frac{C_{HPLC} \cdot 10 \cdot AC}{0.10}$$

where:

C_{HPLC} = Concentration of the sugar as given by HPLC in g/mL

AC = Anhydro correction to calculate the concentration of polymeric sugars from the corresponding concentration of monomeric sugars. It is 0.88 for glucose and mannose and 0.9 for xylose.

The values 10 and 0.10 stand for volume of the sample and grams of cellulose added, respectively.

APPENDIX K

ALL DATA IN SECTION LONG-TERM LIME PRETREATMENT OF POPLAR WOOD

Table K1 shows pretreatment conditions for samples identified in the first column. Use the number in the first column *Std Order* and refer to this table to determine pretreatment conditions in Tables K2 and K3.

Table K1. Pretreatment conditions and experimental design

Std Order	Run Order	Blocks	Temperature (°C)	Time (weeks)	Aeration[(a)]
1	39	1	25	0	1
3	28	1	25	1	1
5	44	1	25	2	1
7	53	1	25	4	1
9	11	1	25	8	1
11	2	1	25	12	1
13	38	1	35	0	1
15	34	1	35	1	1
17	55	1	35	2	1
19	48	1	35	4	1
21	56	1	35	8	1
23	32	1	35	12	1
25	29	1	45	0	1
27	3	1	45	1	1
29	13	1	45	2	1
31	57	1	45	4	1
33	18	1	45	8	1
35	41	1	45	12	1
37	40	1	55	0	1
39	9	1	55	1	1
41	7	1	55	2	1
43	37	1	55	4	1
45	23	1	55	8	1

Table K1. Continued

Std Order	Run Order	Blocks	Temperature (°C)	Time (weeks)	Aeration[(a)]
47	43	1	55	12	1
49	16	1	65	0	1
51	4	1	65	1	1
53	10	1	65	2	1
55	14	1	65	4	1
57	27	1	65	8	1
59	35	1	65	12	1
2	59	1	25	0	2
4	12	1	25	1	2
6	33	1	25	2	2
8	1	1	25	4	2
10	52	1	25	8	2
12	24	1	25	12	2
14	5	1	35	0	2
16	15	1	35	1	2
18	25	1	35	2	2
20	45	1	35	4	2
22	47	1	35	8	2
24	42	1	35	12	2
26	22	1	45	0	2
28	36	1	45	1	2
30	17	1	45	2	2
32	46	1	45	4	2
34	30	1	45	8	2
36	19	1	45	12	2
38	20	1	55	0	2
40	60	1	55	1	2
42	26	1	55	2	2
44	49	1	55	4	2
46	8	1	55	8	2
48	54	1	55	12	2
50	6	1	65	0	2
52	58	1	65	1	2
54	31	1	65	2	2
56	50	1	65	4	2
58	21	1	65	8	2
60	51	1	65	12	2

[(a)] Aeration 1 is for experiments with bubbling air, 2 without air.

Table K2. Degradation of structural components

Std Order	Lignin remaining (g/g raw)	Glucan remaining (g/g raw)	Xylan remaining (g/g raw)
1	1.000	1.000	1.000
3	0.880	0.990	0.882
5	0.840	0.990	0.890
7	0.720	0.980	0.769
9	0.632	0.900	0.626
11	0.569	0.890	0.532
13	1.000	1.000	1.000
15	0.841	1.000	0.933
17	0.800	0.996	0.873
19	0.700	0.989	0.730
21	0.580	0.830	0.600
23	0.560	0.822	0.500
25	1.000	1.000	1.000
27	0.830	0.965	0.838
29	0.740	0.930	0.800
31	0.650	0.900	0.717
33	0.520	0.830	0.554
35	0.439	0.800	0.431
37	1.000	1.000	1.000
39	0.800	0.950	0.879
41	0.730	0.900	0.780
43	0.620	0.848	0.671
45	0.450	0.726	0.522
47	0.380	0.665	0.400
49	1.000	1.000	1.000
51	0.750	0.930	0.869
53	0.668	0.900	0.750
55	0.544	0.856	0.700
57	0.400	0.664	0.500
59	0.287	0.600	0.380
2	1.000	1.000	1.000
4	0.920	0.990	0.897
6	0.880	0.990	0.900
8	0.848	0.990	0.852
10	0.800	0.960	0.789
12	0.750	0.960	0.678

Table 2. Continued

Std Order	Lignin remaining (g/g raw)	Glucan remaining (g/g raw)	Xylan remaining (g/g raw)
14	1.000	1.000	1.000
16	0.880	0.990	0.908
18	0.846	0.990	0.890
20	0.834	0.990	0.839
22	0.732	0.956	0.736
24	0.700	0.932	0.650
26	1.000	1.000	1.000
28	0.850	0.990	0.906
30	0.830	0.990	0.880
32	0.800	0.950	0.867
34	0.754	0.930	0.730
36	0.726	0.900	0.590
38	1.000	1.000	1.000
40	0.800	0.990	0.850
42	0.780	0.990	0.840
44	0.750	0.946	0.800
46	0.680	0.872	0.680
48	0.660	0.795	0.600
50	1.000	1.000	1.000
52	0.800	0.940	0.880
54	0.760	0.940	0.850
56	0.700	0.900	0.800
58	0.689	0.774	0.660
60	0.600	0.700	0.504

Table K3. Enzymatic and overall yields of glucan, xylan and combined

	Enzymatic Hydrolysis		Overall Hydrolysis		
Std Order	Glucan g/g raw	Xylan g/g raw	Glucan g/g raw	Xylan g/g raw	Combined g/g raw
1	0.050	0.260	0.050	0.260	0.103
3	0.255	0.363	0.252	0.320	0.269
5	0.386	0.475	0.382	0.423	0.392
7	0.314	0.383	0.307	0.295	0.304
9	0.342	0.410	0.308	0.257	0.295
11	0.446	0.420	0.397	0.223	0.353
13	0.050	0.260	0.050	0.260	0.103
15	0.280	0.390	0.280	0.364	0.301
17	0.411	0.377	0.409	0.329	0.389
19	0.333	0.464	0.329	0.339	0.332
21	0.423	0.400	0.351	0.240	0.323
23	0.496	0.350	0.407	0.175	0.349
25	0.050	0.260	0.050	0.260	0.103
27	0.245	0.377	0.236	0.316	0.256
29	0.500	0.543	0.465	0.434	0.457
31	0.494	0.623	0.444	0.447	0.445
33	0.760	0.546	0.631	0.302	0.548
35	0.652	0.450	0.522	0.194	0.440
37	0.050	0.260	0.050	0.260	0.103
39	0.428	0.464	0.406	0.408	0.407
41	0.489	0.595	0.440	0.464	0.446
43	0.548	0.595	0.464	0.399	0.448
45	0.771	0.666	0.560	0.348	0.507
47	0.717	0.500	0.477	0.200	0.408
49	0.050	0.260	0.050	0.260	0.103
51	0.428	0.475	0.398	0.413	0.402
53	0.672	0.690	0.605	0.518	0.583
55	0.950	0.835	0.773	0.584	0.726
57	0.895	0.664	0.594	0.332	0.529
59	0.893	0.600	0.536	0.228	0.459
2	0.050	0.260	0.050	0.260	0.103
4	0.203	0.328	0.201	0.294	0.224
6	0.283	0.374	0.281	0.336	0.295

Table K3. Continued.

Std Order	Enzymatic Hydrolysis		Overall Hydrolysis		
	Glucan g/g raw	Xylan g/g raw	Glucan g/g raw	Xylan g/g raw	Combined g/g raw
8	0.198	0.381	0.196	0.324	0.228
10	0.212	0.350	0.204	0.276	0.222
12	0.180	0.330	0.173	0.224	0.186
14	0.050	0.260	0.050	0.260	0.103
16	0.250	0.398	0.248	0.361	0.276
18	0.246	0.466	0.244	0.415	0.287
20	0.272	0.455	0.269	0.382	0.297
22	0.217	0.280	0.207	0.206	0.207
24	0.235	0.255	0.219	0.166	0.206
26	0.050	0.260	0.050	0.260	0.103
28	0.337	0.466	0.334	0.423	0.356
30	0.354	0.474	0.350	0.418	0.367
32	0.385	0.552	0.365	0.479	0.394
34	0.400	0.350	0.372	0.256	0.343
36	0.300	0.276	0.270	0.163	0.243
38	0.050	0.260	0.050	0.260	0.103
40	0.300	0.455	0.297	0.387	0.319
42	0.365	0.497	0.361	0.418	0.375
44	0.375	0.570	0.355	0.456	0.380
46	0.450	0.450	0.392	0.306	0.371
48	0.339	0.576	0.269	0.346	0.288
50	0.050	0.260	0.050	0.260	0.103
52	0.365	0.509	0.343	0.448	0.369
54	0.478	0.539	0.449	0.458	0.452
56	0.479	0.654	0.431	0.523	0.454
58	0.550	0.458	0.426	0.303	0.395
60	0.626	0.576	0.438	0.291	0.401

Table K4. Descriptive statistics for lignin, glucan and xylan pretreatment yields (g component /g component in raw biomass)

Variable	N	Mean	Std Error	Std Deviation	Minimum	Median
Lignin	60	0.7550	0.0221	0.1715	0.2870	0.7568
Glucan	60	0.9201	0.0126	0.0978	0.6000	0.9532
Xylan	60	0.7784	0.0221	0.1712	0.3800	0.8190

Table K5. General linear model for lime consumed vs temperature, time, and aeration

Factor	Levels
Temperature (°C)	25, 35, 45, 55, 65
Time (weeks)	1, 2, 4, 8, 12
Aeration	1, 2

ANOVA

Fuente	DF	SS	SC adjusted.	MS ajust.	F	P-value
Temperature (°C)	4	0.017848	0.017848	0.004462	3.31	0.018
Time (weeks)	5	0.379524	0.379524	0.075905	56.28	0.000
Aeration	1	0.105872	0.105872	0.105872	78.50	0.000
Error	49	0.066088	0.066088	0.001349		
Total	59	0.569331				

S = 0.0367250 R-squared. = 88.39% R-squared.(adjusted) = 86.02%

Table K6. General linear model for lignin pretreatment yields vs temperature, time, and aeration

Factor	Levels
Temperature (°C)	25, 35, 45, 55, 65
Time (weeks)	1, 2, 4, 8, 12
Aeration	1, 2

ANOVA

Fuente	DF	SS	SC adjusted.	MS ajust.	F	P-value
Temperature (°C)	4	0.14119	0.14119	0.03530	9.57	0.000
Time (weeks)	5	1.21532	1.21532	0.24306	65.89	0.000
Aeration	1	0.19715	0.19715	0.19715	53.44	0.000
Error	49	0.18076	0.18076	0.00369		
Total	59	1.73442				

S = 0.0607369 R-squared. = 89.58% R-squared.(adjusted) = 87.45%

Table K7. General linear model for glucan pretreatment yields vs temperature, time, and aeration

Factor	Levels
Temperature (°C)	25, 35, 45, 55, 65
Time (weeks)	1, 2, 4, 8, 12
Aeration	1, 2

ANOVA

Fuente	DF	SS	SC adjusted.	MS ajust.	F	P-value
Temperature (°C)	4	0.117963	0.117963	0.029491	13.10	0.000
Time (weeks)	5	0.298312	0.298312	0.059662	26.50	0.000
Aeration	1	0.037838	0.037838	0.037838	16.81	0.000
Error	49	0.110318	0.110318	0.002251		
Total	59	0.564431				

S = 0.0474487 R-squared. = 80.46% R-squared.(adjusted) = 76.47%

Table K8. General linear model for xylan pretreatment yields vs temperature, time, and aeration

Factor	Levels
Temperature (°C)	25, 35, 45, 55, 65
Time (weeks)	1, 2, 4, 8, 12
Aeration	1, 2

ANOVA

Fuente	DF	SS	SC adjusted.	MS ajust.	F	P-value
Temperature (°C)	4	0.05252	0.05252	0.01313	6.40	0.000
Time (weeks)	5	1.47540	1.47540	0.29508	143.80	0.000
Aeration	1	0.09996	0.09996	0.09996	48.72	0.000
Error	49	0.10055	0.10055	0.00205		
Total	59	1.72842				

S = 0.0452985 R-squared. = 94.18% R-squared.(adjusted) = 93.00%

Table K9. General linear model for glucan enzymatic yields vs temperature, time, and aeration

Factor	Levels
Temperature (°C)	25, 35, 45, 55, 65
Time (weeks)	1, 2, 4, 8, 12
Aeration	1, 2

ANOVA

Fuente	DF	SS	SC adjusted.	MS ajust.	F	P-value
Temperature (°C)	4	6415.7	6415.7	1603.9	15.04	0.000
Time (weeks)	5	14345.2	14345.2	2869.0	26.90	0.000
Aeration	1	3650.2	3650.2	3650.2	34.22	0.000
Error	49	5227.1	5227.1	106.7		
Total	59	29638.2				

S = 10.3283 R-squared. = 82.36% R-squared.(adjusted) = 78.76%

Table K10. General linear model for xylan enzymatic yields vs temperature, time, and aeration

Factor	Levels
Temperature (°C)	25, 35, 45, 55, 65
Time (weeks)	1, 2, 4, 8, 12
Aeration	1, 2

ANOVA

Fuente	DF	SS	SC adjusted.	MS ajust.	F	P-value
Temperature (°C)	4	3055.18	3055.18	763.79	15.78	0.000
Time (weeks)	5	4938.55	4938.55	987.71	20.41	0.000
Aeration	1	446.21	446.21	446.21	9.22	0.004
Error	49	2371.09	2371.09	48.39		
Total	59	10811.02				

S = 6.95626 R-squared. = 78.07% R-squared.(adjusted) = 73.59%

APPENDIX L

ALL DATA IN SECTION SELECTIVITY AND DELIGNIFICATION KINETICS FOR SHORT-TERM LIME PRETREATMENT OF POPLAR WOOD. PART I: CONSTANT- PRESSURE

Table L1. Lignin measured vs. lignin calculated and model assessment for Model 1 and all pretreatment conditions (α=0.05).

Pretreatment conditions			Measured lignin yield	Calculated			Model assessment	
Time (min)	Temp (K)	Pressure[(a)] (bar)		Fast degrading lignin	Slow degrading lignin	Lignin total	Residuals	Y_L CI[(b)]
0	383	6.47	1.000	0.384	0.616	1.000	0.000	0.000
120	383	6.47	0.860	0.347	0.603	0.950	-0.090	0.024
240	383	6.47	0.850	0.314	0.590	0.904	-0.054	0.043
600	383	6.47	0.783	0.232	0.552	0.784	-0.001	0.077
0	383	13.4	1.000	0.384	0.616	1.000	0.000	0.000
120	383	13.4	0.822	0.324	0.588	0.912	-0.090	0.030
240	383	13.4	0.788	0.274	0.561	0.835	-0.047	0.049
360	383	13.4	0.704	0.232	0.535	0.766	-0.062	0.061
600	383	13.4	0.680	0.165	0.486	0.652	0.028	0.069
0	383	20.3	1.000	0.384	0.616	1.000	0.000	0.000
120	383	20.3	0.870	0.306	0.573	0.879	-0.009	0.037
240	383	20.3	0.840	0.244	0.532	0.776	0.064	0.057
360	383	20.3	0.790	0.194	0.495	0.689	0.101	0.067
600	383	20.3	0.750	0.124	0.427	0.551	0.199	0.069
0	403	4.29	1.000	0.384	0.616	1.000	0.000	0.000
120	403	4.29	0.810	0.248	0.599	0.847	-0.037	0.046
360	403	4.29	0.700	0.104	0.565	0.669	0.031	0.057
600	403	4.29	0.630	0.043	0.534	0.577	0.053	0.049
0	403	11.2	1.000	0.384	0.616	1.000	0.000	0.000
120	403	11.2	0.700	0.162	0.570	0.731	-0.031	0.076
240	403	11.2	0.480	0.068	0.527	0.595	-0.115	0.063
360	403	11.2	0.400	0.029	0.487	0.516	-0.116	0.044
600	403	11.2	0.370	0.005	0.416	0.421	-0.051	0.039
0	403	18.1	1.000	0.384	0.616	1.000	0.000	0.000
120	403	18.1	0.558	0.113	0.541	0.654	-0.096	0.092
240	403	18.1	0.400	0.034	0.475	0.509	-0.108	0.064
600	403	18.1	0.250	0.001	0.322	0.322	-0.072	0.063

Table L1. Continued

Pretreatment conditions			Measured lignin yield	Calculated			Model assessment	
Time (min)	Temp (K)	Pressure[a] (bar)		Fast degrading lignin	Slow degrading lignin	Lignin total	Residuals	Y_L CI[b]
0	433	1.72	1.000	0.384	0.616	1.000	0.000	0.000
120	433	1.72	0.850	0.036	0.599	0.635	0.214	0.065
240	433	1.72	0.650	0.003	0.583	0.587	0.063	0.062
600	433	1.72	0.630	0.000	0.537	0.537	0.093	0.069
0	433	8.62	1.000	0.384	0.616	1.000	0.000	0.000
120	433	8.62	0.530	0.000	0.530	0.531	-0.001	0.066
240	433	8.62	0.420	0.000	0.457	0.457	-0.037	0.068
600	433	8.62	0.330	0.000	0.292	0.292	0.038	0.103
0	433	15.5	1.000	0.384	0.616	1.000	0.000	0.000
120	433	15.5	0.470	0.000	0.467	0.467	0.003	0.090
240	433	15.5	0.270	0.000	0.353	0.353	-0.083	0.113
600	433	15.5	0.230	0.000	0.154	0.154	0.076	0.119
0	453	0.031	1.000	0.384	0.616	1.000	0.000	0.000
120	453	0.031	0.850	0.223	0.616	0.839	0.007	0.089
240	453	0.031	0.700	0.129	0.615	0.745	-0.049	0.102
600	453	0.031	0.670	0.025	0.614	0.639	0.028	0.071
0	453	4.77	1.000	0.384	0.616	1.000	0.000	0.000
120	453	4.77	0.500	0.000	0.536	0.536	-0.036	0.071
240	453	4.77	0.419	0.000	0.467	0.467	-0.048	0.087
600	453	4.77	0.260	0.000	0.308	0.308	-0.048	0.149
0	453	11.7	1.000	0.384	0.616	1.000	0.000	0.000
120	453	11.7	0.450	0.000	0.432	0.432	0.018	0.118
240	453	11.7	0.350	0.000	0.303	0.303	0.047	0.156
600	453	11.7	0.180	0.000	0.104	0.104	0.076	0.134

[a] Partial oxygen pressure

[b] Confidence intervals for response variable.

Table L2. Glucan measured vs. glucan calculated and model assessment for Model 1 and all pretreatment conditions (α=0.05).

Pretreatment conditions			Measured glucan yield	Calculated			Model assessment	
Time (min)	Temp (K)	Pressure[a] (bar)		Fast degrading glucan	Slow degrading glucan	Glucan total	Residuals	Y_L CI[b]
0	383	6.47	1.000	0.110	0.890	1.000	0.000	0.000
120	383	6.47	1.000	0.104	0.889	0.993	0.006	0.008
240	383	6.47	0.990	0.098	0.888	0.986	0.001	0.015
600	383	6.47	0.970	0.082	0.884	0.966	-0.003	0.035
0	383	13.4	1.000	0.110	0.890	1.000	0.000	0.000
120	383	13.4	0.994	0.103	0.887	0.990	0.002	0.007
240	383	13.4	0.985	0.096	0.884	0.980	0.001	0.014
360	383	13.4	0.978	0.089	0.881	0.971	0.001	0.020
600	383	13.4	0.965	0.078	0.876	0.954	0.003	0.032
0	383	20.3	1.000	0.110	0.890	1.000	0.000	0.000
120	383	20.3	0.991	0.102	0.886	0.987	0.005	0.009
240	383	20.3	0.980	0.094	0.881	0.975	0.008	0.018
360	383	20.3	0.980	0.087	0.876	0.964	0.021	0.026
600	383	20.3	0.960	0.075	0.867	0.942	0.028	0.042
0	403	4.29	1.000	0.110	0.890	1.000	0.000	0.000
120	403	4.29	1.000	0.093	0.887	0.980	0.015	0.023
360	403	4.29	0.970	0.067	0.882	0.949	0.013	0.059
600	403	4.29	0.970	0.048	0.876	0.924	0.038	0.084
0	403	11.2	1.000	0.110	0.890	1.000	0.000	0.000
120	403	11.2	0.970	0.089	0.882	0.971	-0.009	0.021
240	403	11.2	0.930	0.072	0.874	0.946	-0.049	0.039
360	403	11.2	0.910	0.058	0.866	0.925	-0.030	0.054
600	403	11.2	0.860	0.038	0.851	0.889	-0.045	0.077
0	403	18.1	1.000	0.110	0.890	1.000	0.000	0.000
120	403	18.1	0.960	0.087	0.877	0.963	-0.007	0.022
240	403	18.1	0.880	0.068	0.863	0.932	-0.074	0.041
600	403	18.1	0.800	0.033	0.825	0.858	-0.046	0.083
0	433	1.72	1.000	0.110	0.890	1.000	0.000	0.000
120	433	1.72	0.980	0.059	0.885	0.944	0.036	0.081
240	433	1.72	0.930	0.031	0.880	0.912	0.027	0.116
600	433	1.72	0.900	0.005	0.865	0.870	0.066	0.110
0	433	8.6	1.000	0.110	0.890	1.000	0.000	0.000
120	433	8.6	0.940	0.043	0.861	0.904	0.015	0.078
240	433	8.6	0.860	0.017	0.832	0.849	-0.006	0.109
600	433	8.6	0.820	0.001	0.753	0.754	0.073	0.097
0	433	15.5	1.000	0.110	0.890	1.000	0.000	0.000
120	433	15.5	0.850	0.037	0.835	0.872	-0.039	0.079

Table L2. Continued

Pretreatment conditions			Measured glucan yield	Calculated			Model assessment	
Time (min)	Temp (K)	Pressure[(a)] (bar)		Fast degrading glucan	Slow degrading glucan	Glucan total	Residuals	Y_L CI[(b)]
240	433	15.5	0.800	0.013	0.783	0.795	0.002	0.113
600	433	15.5	0.650	0.000	0.645	0.646	0.050	0.107
0	453	0.031	1.000	0.110	0.890	1.000	0.000	0.000
120	453	0.031	0.930	0.061	0.890	0.951	0.004	0.073
240	453	0.031	0.910	0.034	0.890	0.924	-0.031	0.072
600	453	0.031	0.880	0.006	0.889	0.895	-0.019	0.056
0	453	4.77	1.000	0.110	0.890	1.000	0.000	0.000
120	453	4.77	0.850	0.014	0.851	0.865	-0.022	0.124
240	453	4.77	0.750	0.002	0.813	0.815	-0.054	0.108
600	453	4.77	0.690	0.000	0.710	0.710	-0.018	0.040
0	453	11.7	1.000	0.110	0.890	1.000	0.000	0.000
120	453	11.7	0.790	0.009	0.788	0.797	-0.028	0.121
240	453	11.7	0.720	0.001	0.698	0.699	0.017	0.106
600	453	11.7	0.480	0.000	0.485	0.485	-0.018	0.069

(a) Partial oxygen pressure

(b) Confidence intervals for response variable.

Table L3. Xylan measured vs. xylan calculated and model assessment for Model 1 and all pretreatment conditions (α=0.05).

Pretreatment conditions			Measured xylan yield	Calculated			Model assessment	
Time (min)	Temp (K)	Pressure[a] (bar)		Fast degrading xylan	Slow degrading xylan	Xylan total	Residuals	Y_L CI[b]
0	383	6.47	1.00	0.37	0.64	1.00	0.000	0.000
120	383	6.47	0.98	0.34	0.63	0.97	0.018	0.013
240	383	6.47	0.82	0.32	0.63	0.95	0.005	0.025
600	383	6.47	0.84	0.26	0.63	0.89	0.029	0.051
0	383	13.4	0.00	0.37	0.64	1.00	0.000	0.000
120	383	13.4	0.97	0.33	0.63	0.97	0.007	0.015
240	383	13.4	0.85	0.30	0.63	0.94	0.011	0.027
360	383	13.4	0.96	0.28	0.63	0.91	0.023	0.037
600	383	13.4	0.86	0.23	0.63	0.86	0.053	0.051
0	383	20.3	0.00	0.37	0.64	1.00	0.000	0.000
120	383	20.3	0.92	0.33	0.63	0.96	-0.007	0.016
240	383	20.3	0.87	0.30	0.63	0.93	0.014	0.028
360	383	20.3	0.89	0.27	0.63	0.90	0.031	0.038
600	383	20.3	0.85	0.21	0.63	0.84	0.057	0.050
0	403	4.29	1.00	0.37	0.64	1.00	0.000	0.000
120	403	4.29	0.90	0.29	0.63	0.93	-0.023	0.023
360	403	4.29	0.85	0.19	0.63	0.82	0.029	0.037
600	403	4.29	0.80	0.12	0.63	0.76	0.037	0.035
0	403	11.2	1.00	0.37	0.64	1.00	0.000	0.000
120	403	11.2	0.82	0.27	0.63	0.90	-0.058	0.040
240	403	11.2	0.73	0.20	0.63	0.83	-0.072	0.046
360	403	11.2	0.75	0.15	0.63	0.77	-0.003	0.041
600	403	11.2	0.73	0.08	0.62	0.70	0.032	0.035
0	403	18.1	1.00	0.37	0.64	1.00	0.000	0.000
120	403	18.1	0.73	0.26	0.63	0.88	-0.118	0.056
240	403	18.1	0.68	0.18	0.62	0.80	-0.083	0.056
600	403	18.1	0.65	0.06	0.61	0.67	-0.010	0.049
0	433	1.72	1.00	0.37	0.64	1.00	0.000	0.000
120	433	1.72	0.90	0.16	0.63	0.79	0.161	0.059
240	433	1.72	0.83	0.07	0.63	0.70	0.133	0.046
600	433	1.72	0.70	0.01	0.62	0.62	0.029	0.052
0	433	8.62	1.00	0.37	0.64	1.00	0.000	0.000
120	433	8.62	0.71	0.09	0.60	0.69	0.053	0.040
240	433	8.62	0.54	0.02	0.57	0.59	-0.067	0.040
600	433	8.62	0.56	0.00	0.49	0.49	0.075	0.069
0	433	15.5	1.00	0.37	0.64	1.00	0.000	0.000
120	433	15.5	0.57	0.06	0.56	0.63	-0.034	0.044

Table L3. Continued

Pretreatment conditions			Measured xylan yield	Calculated			Model assessment	
Time (min)	Temp (K)	Pressure[(a)] (bar)		Fast degrading xylan	Slow degrading xylan	Xylan total	Residuals	Y_L CI[(b)]
240	433	15.5	0.48	0.01	0.50	0.51	-0.036	0.053
600	433	15.5	0.32	0.00	0.35	0.35	-0.003	0.088
0	453	0.031	1.00	0.37	0.64	1.00	0.000	0.000
120	453	0.031	0.80	0.22	0.63	0.85	-0.021	0.095
240	453	0.031	0.70	0.13	0.63	0.76	-0.051	0.074
600	453	0.031	0.68	0.03	0.63	0.66	-0.027	0.058
0	453	4.77	1.00	0.37	0.64	1.00	0.000	0.000
120	453	4.77	0.55	0.02	0.56	0.58	-0.058	0.046
240	453	4.77	0.41	0.00	0.50	0.50	-0.113	0.058
600	453	4.77	0.36	0.00	0.35	0.35	0.026	0.099
0	453	11.7	1.00	0.37	0.64	1.00	0.000	0.000
120	453	11.7	0.40	0.00	0.42	0.43	-0.046	0.069
240	453	11.7	0.25	0.00	0.28	0.28	-0.033	0.081
600	453	11.7	0.27	0.00	0.08	0.08	0.198	0.051

(a) Partial oxygen pressure

(b) Confidence intervals for response variable.

Table L4. Differential and integral selectivity

Pretreatment conditions			Selectivity			
Time	Pressure[(a)]	Temperature	Differential		Integral	
(min)	(bar)	(K)	Glucan	Xylan	Glucan	Xylan
0	6.47	383	6.860	1.997	6.860	1.997
120	6.47	383	6.645	1.979	6.752	1.988
240	6.47	383	6.441	1.964	6.650	1.980
600	6.47	383	5.894	1.933	6.373	1.961
0	13.4	383	8.978	2.757	8.978	2.757
120	13.4	383	8.294	2.651	8.633	2.704
240	13.4	383	7.680	2.558	8.316	2.656
360	13.4	383	7.129	2.476	8.026	2.614
600	13.4	383	6.191	2.344	7.515	2.543
0	20.3	383	10.189	3.324	10.189	3.324
120	20.3	383	9.024	3.106	9.596	3.215
240	20.3	383	8.028	2.918	9.070	3.118
360	20.3	383	7.174	2.756	8.602	3.034
600	20.3	383	5.809	2.499	7.809	2.895
0	4.29	403	17.191	2.344	17.191	2.344
120	4.29	403	11.981	1.961	14.422	2.151
360	4.29	403	6.207	1.463	10.685	1.885
600	4.29	403	3.652	1.235	8.420	1.731
0	11.2	403	18.074	3.367	18.074	3.367
120	11.2	403	8.990	2.184	12.931	2.742
240	11.2	403	5.006	1.582	9.874	2.365
360	11.2	403	3.228	1.321	7.968	2.139
600	11.2	403	1.997	1.348	5.855	1.932
0	18.1	403	15.401	4.018	15.401	4.018
120	18.1	403	6.075	2.146	9.884	2.988
240	18.1	403	3.097	1.470	7.154	2.477
600	18.1	403	1.440	1.549	4.158	2.031
0	1.72	433	22.951	3.071	22.951	3.071
120	1.72	433	2.995	0.752	9.843	1.760
240	1.72	433	0.838	0.388	6.056	1.375
600	1.72	433	0.845	1.715	3.406	1.229
0	8.62	433	44.305	5.274	44.305	5.274
120	8.62	433	1.393	0.518	7.534	1.506
240	8.62	433	1.339	1.146	4.652	1.330
600	8.62	433	1.210	1.667	2.887	1.383
0	15.5	433	43.077	6.247	43.077	6.247
120	15.5	433	1.408	0.734	5.419	1.424
240	15.5	433	1.210	1.257	3.497	1.322
600	15.5	433	0.752	1.021	2.176	1.303

Table L4. Continued

Pretreatment conditions			Selectivity			
Time	Pressure[(a)]	Temperature	Differential		Integral	
(min)	(bar)	(K)	Glucan	Xylan	Glucan	Xylan
0	0.031	453	2.658	1.053	2.658	1.053
120	0.031	453	2.247	1.055	2.459	1.054
240	0.031	453	1.901	1.057	2.302	1.054
600	0.031	453	1.168	1.077	2.017	1.057
0	4.77	453	60.542	6.246	60.542	6.246
120	4.77	453	0.904	0.638	4.542	1.105
240	4.77	453	1.159	1.054	3.136	1.071
600	4.77	453	1.776	1.028	2.485	1.068
0	11.7	453	77.288	7.911	77.288	7.911
120	11.7	453	1.157	0.796	3.605	0.994
240	11.7	453	1.088	0.938	2.563	0.972
600	11.7	453	0.697	1.092	1.840	0.978

(a) Partial oxygen pressure

APPENDIX M

ALL DATA IN SECTION SELECTIVITY AND DELIGNIFICATION KINETICS FOR SHORT-TERM LIME PRETREATMENT OF POPLAR WOOD. PART II: VARYING- PRESSURE

Table M1. Lignin measured vs. lignin calculated and model assessment for Model 1 and all pretreatment conditions (α=0.05).

Pretreatment conditions			Measured lignin yield	Calculated			Model assessment	
Time (min)	Temp (K)	Pressure[(a)] ratio		Fast degrading lignin	Slow degrading lignin	Lignin total	Residuals	Y_L CI[(b)]
0	403	0.106	1.000	0.586	0.414	1.000	0.000	0.000
60	403	0.106	0.918	0.516	0.412	0.928	-0.011	0.016
240	403	0.106	0.750	0.354	0.406	0.759	-0.010	0.042
360	403	0.106	0.650	0.275	0.401	0.676	-0.027	0.048
600	403	0.106	0.600	0.166	0.393	0.559	0.041	0.047
0	403	0.198	1.000	0.586	0.414	1.000	0.000	0.000
60	403	0.198	0.880	0.492	0.409	0.901	-0.020	0.017
240	403	0.198	0.700	0.292	0.393	0.685	0.017	0.036
360	403	0.198	0.600	0.206	0.383	0.589	0.013	0.035
600	403	0.198	0.500	0.103	0.364	0.467	0.034	0.031
0	403	0.291	1.000	0.586	0.414	1.000	0.000	0.000
60	403	0.291	0.848	0.474	0.405	0.879	-0.029	0.022
240	403	0.291	0.620	0.251	0.379	0.630	-0.006	0.040
360	403	0.291	0.585	0.165	0.362	0.527	0.060	0.035
600	403	0.291	0.461	0.071	0.331	0.402	0.056	0.040
0	403	0.383	1.000	0.586	0.414	1.000	0.000	0.000
60	403	0.383	0.781	0.459	0.401	0.860	-0.076	0.029
240	403	0.383	0.565	0.221	0.363	0.584	-0.016	0.045
360	403	0.383	0.529	0.136	0.340	0.475	0.052	0.041
600	403	0.383	0.450	0.051	0.298	0.349	0.092	0.059
0	433	0.106	1.000	0.586	0.414	0.414	0.000	0.000
60	433	0.106	0.791	0.423	0.410	0.410	-0.040	0.032
240	433	0.106	0.500	0.160	0.396	0.396	-0.051	0.043
360	433	0.106	0.469	0.084	0.387	0.387	0.003	0.038
600	433	0.106	0.398	0.023	0.370	0.370	0.010	0.040
0	433	0.198	1.000	0.586	0.414	0.414	0.000	0.000
60	433	0.198	0.759	0.374	0.403	0.403	-0.012	0.026

Table M1. Continued

Pretreatment conditions			Measured lignin yield	Calculated			Model assessment	
Time (min)	Temp (K)	Pressure[(a)] ratio		Fast degrading lignin	Slow degrading lignin	Lignin total	Residuals	Y_L CI[(b)]
240	433	0.198	0.368	0.098	0.372	0.372	-0.095	0.026
360	433	0.198	0.340	0.040	0.352	0.352	-0.048	0.028
600	433	0.198	0.287	0.007	0.315	0.315	-0.035	0.037
0	433	0.291	1.000	0.586	0.414	0.414	0.000	0.000
60	433	0.291	0.600	0.340	0.395	0.395	-0.126	0.032
240	433	0.291	0.350	0.066	0.344	0.344	-0.057	0.030
360	433	0.291	0.260	0.022	0.313	0.313	-0.078	0.029
600	433	0.291	0.150	0.003	0.259	0.259	-0.121	0.041
0	433	0.383	1.000	0.586	0.414	0.414	0.000	0.000
60	433	0.383	0.660	0.313	0.387	0.387	-0.029	0.042
240	433	0.383	0.253	0.047	0.314	0.314	-0.112	0.043
360	433	0.383	0.269	0.014	0.274	0.274	-0.029	0.046
0	453	0.106	1.000	0.586	0.414	0.414	0.000	0.000
60	453	0.106	0.802	0.332	0.407	0.407	0.069	0.056
360	453	0.106	0.468	0.019	0.373	0.373	0.082	0.049
600	453	0.106	0.350	0.002	0.348	0.348	0.008	0.057
0	453	0.198	1.000	0.586	0.414	0.414	0.000	0.000
60	453	0.198	0.700	0.267	0.397	0.397	0.047	0.045
360	453	0.198	0.350	0.005	0.322	0.322	0.025	0.041
600	453	0.198	0.300	0.000	0.272	0.272	0.028	0.063
0	453	0.291	1.000	0.586	0.414	0.414	0.000	0.000
60	453	0.291	0.634	0.225	0.385	0.385	0.037	0.047
240	453	0.291	0.400	0.013	0.310	0.310	0.075	0.051
360	453	0.291	0.250	0.002	0.268	0.268	-0.026	0.044
600	453	0.291	0.200	0.000	0.201	0.201	-0.010	0.060
0	453	0.383	1.000	0.586	0.414	0.414	0.000	0.000
60	453	0.383	0.649	0.195	0.372	0.372	0.095	0.054
360	453	0.383	0.300	0.001	0.218	0.218	0.066	0.065
600	453	0.383	0.190	0.000	0.142	0.142	0.031	0.068

(a) Ratio kg oxygen/kg biomass

(b) Confidence intervals for response variable.

Table M2. Glucan measured vs. glucan calculated and model assessment for Model 1 and all pretreatment conditions (α=0.05).

Pretreatment conditions			Measured glucan yield	Calculated			Model assessment	
Time (min)	Temp (K)	Pressure[a] ratio		Fast degrading glucan	Slow degrading glucan	Glucan total	Residuals	Y_L CI[b]
0	403	0.106	1.000	0.877	0.123	1.000	0.000	0.000
60	403	0.106	1.010	0.877	0.123	1.000	0.011	0.001
240	403	0.106	0.980	0.876	0.122	0.998	-0.018	0.002
360	403	0.106	0.980	0.875	0.122	0.997	-0.017	0.003
600	403	0.106	0.940	0.874	0.122	0.996	-0.054	0.005
0	403	0.198	1.000	0.877	0.123	1.000	0.000	0.000
60	403	0.198	1.000	0.877	0.120	0.996	0.006	0.005
240	403	0.198	0.970	0.875	0.111	0.986	-0.009	0.018
360	403	0.198	0.950	0.874	0.106	0.980	-0.019	0.025
600	403	0.198	0.930	0.872	0.096	0.968	-0.022	0.035
0	403	0.291	1.000	0.877	0.123	1.000	0.000	0.000
60	403	0.291	0.950	0.877	0.100	0.976	-0.021	0.032
240	403	0.291	0.910	0.875	0.054	0.928	-0.007	0.058
360	403	0.291	0.900	0.873	0.035	0.909	0.003	0.052
600	403	0.291	0.850	0.871	0.015	0.886	-0.027	0.033
0	403	0.383	1.000	0.877	0.123	1.000	0.000	0.000
60	403	0.383	0.920	0.876	0.047	0.924	-0.003	0.079
240	403	0.383	0.900	0.874	0.003	0.877	0.026	0.029
360	403	0.383	0.900	0.872	0.000	0.873	0.031	0.031
600	403	0.383	0.830	0.869	0.000	0.869	-0.036	0.031
0	433	0.106	1.000	0.877	0.123	1.000	0.000	0.000
60	433	0.106	1.000	0.873	0.122	0.995	0.005	0.003
240	433	0.106	1.000	0.860	0.122	0.982	0.018	0.011
360	433	0.106	0.980	0.852	0.121	0.973	0.007	0.017
600	433	0.106	0.950	0.835	0.120	0.955	-0.005	0.028
0	433	0.198	1.000	0.877	0.123	1.000	0.000	0.000
60	433	0.198	0.990	0.871	0.114	0.984	0.007	0.017
240	433	0.198	0.960	0.852	0.090	0.942	0.021	0.052
360	433	0.198	0.950	0.839	0.077	0.916	0.038	0.065
600	433	0.198	0.880	0.815	0.057	0.871	0.013	0.079
0	433	0.291	1.000	0.877	0.123	1.000	0.000	0.000
60	433	0.291	0.990	0.869	0.064	0.933	0.050	0.075
240	433	0.291	0.890	0.844	0.009	0.854	0.032	0.062
360	433	0.291	0.840	0.828	0.003	0.831	0.007	0.039
600	433	0.291	0.810	0.797	0.000	0.798	0.011	0.040
0	433	0.383	1.000	0.877	0.123	1.000	0.000	0.000

Table M2. Continued

Pretreatment conditions			Measured glucan yield	Calculated			Model assessment	
Time (min)	Temp (K)	Pressure[a] (ratio		Fast degrading glucan	Slow degrading glucan	Glucan total	Residuals	Y_L CI[b]
60	433	0.383	0.910	0.867	0.006	0.874	0.027	0.079
240	433	0.383	0.890	0.838	0.000	0.838	0.054	0.025
360	433	0.383	0.800	0.819	0.000	0.819	-0.018	0.029
0	453	0.106	1.000	0.877	0.123	1.000	0.000	0.000
60	453	0.106	1.000	0.858	0.122	0.980	0.020	0.006
360	453	0.106	0.860	0.770	0.119	0.889	-0.027	0.032
600	453	0.106	0.840	0.705	0.117	0.822	0.021	0.049
0	453	0.198	1.000	0.877	0.123	1.000	0.000	0.000
60	453	0.198	0.850	0.849	0.105	0.954	-0.106	0.029
360	453	0.198	0.710	0.719	0.049	0.769	-0.062	0.087
600	453	0.198	0.740	0.630	0.027	0.657	0.080	0.084
0	453	0.291	1.000	0.877	0.123	1.000	0.000	0.000
60	453	0.291	0.850	0.841	0.035	0.875	-0.043	0.103
240	453	0.291	0.700	0.740	0.001	0.741	-0.041	0.036
360	453	0.291	0.600	0.679	0.000	0.680	-0.077	0.024
600	453	0.291	0.550	0.573	0.000	0.573	-0.020	0.033
0	453	0.383	1.000	0.877	0.123	1.000	0.000	0.000
60	453	0.383	0.800	0.834	0.000	0.834	-0.036	0.050
360	453	0.383	0.650	0.645	0.000	0.645	0.008	0.032
600	453	0.383	0.570	0.526	0.000	0.526	0.046	0.046

(a) Ratio kg oxygen/kg biomass

(b) Confidence intervals for response variable.

Table M3. Xylan measured vs. xylan calculated and model assessment for Model 1 and all pretreatment conditions (α=0.05).

Pretreatment conditions			Measured xylan yield	Calculated			Model assessment	
Time (min)	Temp (K)	Pressure[a] ratio		Fast degrading xylan	Slow degrading xylan	Xylan total	Residuals	Y_L CI[b]
0	403	0.106	1.000	0.447	0.553	1.000	0.000	0.000
60	403	0.106	0.980	0.432	0.552	0.984	-0.003	0.003
240	403	0.106	0.960	0.388	0.549	0.937	0.023	0.011
360	403	0.106	0.900	0.361	0.547	0.909	-0.008	0.015
600	403	0.106	0.830	0.314	0.544	0.858	-0.026	0.022
0	403	0.198	1.000	0.447	0.553	1.000	0.000	0.000
60	403	0.198	0.950	0.426	0.552	0.977	-0.027	0.004
240	403	0.198	0.900	0.367	0.548	0.915	-0.016	0.011
360	403	0.198	0.880	0.333	0.546	0.879	0.001	0.014
600	403	0.198	0.840	0.273	0.541	0.815	0.026	0.018
0	403	0.291	1.000	0.447	0.553	1.000	0.000	0.000
60	403	0.291	0.950	0.421	0.551	0.973	-0.023	0.005
240	403	0.291	0.890	0.352	0.547	0.899	-0.009	0.013
360	403	0.291	0.860	0.312	0.545	0.857	0.003	0.016
600	403	0.291	0.800	0.245	0.540	0.785	0.016	0.020
0	403	0.383	1.000	0.447	0.553	1.000	0.000	0.000
60	403	0.383	0.950	0.417	0.551	0.969	-0.019	0.006
240	403	0.383	0.840	0.339	0.547	0.886	-0.046	0.017
360	403	0.383	0.830	0.295	0.544	0.839	-0.009	0.020
600	403	0.383	0.780	0.223	0.538	0.761	0.019	0.024
0	433	0.106	1.000	0.447	0.553	1.000	0.000	0.000
60	433	0.106	0.980	0.399	0.545	0.944	0.036	0.009
240	433	0.106	0.840	0.283	0.524	0.806	0.034	0.025
360	433	0.106	0.700	0.225	0.510	0.735	-0.034	0.030
600	433	0.106	0.600	0.142	0.483	0.625	-0.026	0.039
0	433	0.198	1.000	0.447	0.553	1.000	0.000	0.000
60	433	0.198	0.950	0.382	0.543	0.925	0.025	0.012
240	433	0.198	0.800	0.237	0.515	0.752	0.048	0.026
360	433	0.198	0.650	0.172	0.498	0.670	-0.020	0.031
600	433	0.198	0.580	0.091	0.464	0.555	0.023	0.046
0	433	0.291	1.000	0.447	0.553	1.000	0.000	0.000
60	433	0.291	0.920	0.368	0.541	0.910	0.010	0.015
240	433	0.291	0.750	0.206	0.509	0.715	0.035	0.030
360	433	0.291	0.600	0.140	0.488	0.628	-0.029	0.035
600	433	0.291	0.550	0.064	0.450	0.514	0.033	0.055
0	433	0.383	1.000	0.447	0.553	1.000	0.000	0.000
60	433	0.383	0.880	0.357	0.540	0.898	-0.018	0.019

Table M3. Continued

Pretreatment conditions			Measured xylan yield	Calculated			Model assessment	
Time (min)	Temp (K)	Pressure[(a)] ratio		Fast degrading xylan	Slow degrading xylan	Xylan total	Residuals	Y_L CI[(b)]
240	433	0.383	0.680	0.183	0.504	0.686	-0.007	0.034
360	433	0.383	0.580	0.117	0.481	0.598	-0.019	0.039
0	453	0.106	1.000	0.447	0.553	1.000	0.000	0.000
60	453	0.106	0.920	0.355	0.527	0.882	0.039	0.021
360	453	0.106	0.480	0.113	0.414	0.527	-0.046	0.062
600	453	0.106	0.400	0.045	0.342	0.387	0.011	0.100
0	453	0.198	1.000	0.447	0.553	1.000	0.000	0.000
60	453	0.198	0.900	0.325	0.519	0.844	0.056	0.026
360	453	0.198	0.430	0.066	0.380	0.446	-0.014	0.066
600	453	0.198	0.350	0.018	0.296	0.315	0.034	0.112
0	453	0.291	1.000	0.447	0.553	1.000	0.000	0.000
60	453	0.291	0.840	0.303	0.514	0.817	0.024	0.031
240	453	0.291	0.450	0.094	0.412	0.507	-0.053	0.052
360	453	0.291	0.380	0.043	0.356	0.399	-0.017	0.070
0	453	0.383	1.000	0.447	0.553	1.000	0.000	0.000
60	453	0.383	0.800	0.285	0.509	0.795	0.007	0.035
360	453	0.383	0.350	0.030	0.337	0.368	-0.015	0.075
600	453	0.383	0.250	0.005	0.243	0.248	0.001	0.135

(a) Ratio kg oxygen/kg biomass

(b) Confidence intervals for response variable.

Table M4. Differential and integral selectivity

Pretreatment conditions			Selectivity			
Time	Pressure[(a)]	Temperature	Differential		Integral	
(min)	ratio	(K)	Glucan	Xylan	Glucan	Xylan
0	0.106	403	173.4	4.53	173.4	4.53
60	0.106	403	153.5	4.15	163.2	4.34
240	0.106	403	106.9	3.19	137.4	3.84
360	0.106	403	84.3	2.69	123.3	3.56
600	0.106	403	52.9	1.92	100.9	3.10
0	0.198	403	30.0	4.63	30.0	4.63
60	0.198	403	26.0	4.11	27.9	4.37
240	0.198	403	17.0	2.91	22.9	3.72
360	0.198	403	13.0	2.34	20.4	3.39
600	0.198	403	7.82	1.56	16.6	2.88
0	0.291	403	5.11	4.72	5.11	4.72
60	0.291	403	5.13	4.11	5.12	4.41
240	0.291	403	5.24	2.75	5.16	3.67
360	0.291	403	5.37	2.14	5.19	3.30
600	0.291	403	5.78	1.40	5.25	2.78
0	0.383	403	1.33	4.81	1.33	4.81
60	0.383	403	2.73	4.11	1.84	4.45
240	0.383	403	19.5	2.64	3.38	3.64
360	0.383	403	37.5	2.02	4.13	3.26
600	0.383	403	27.7	1.32	4.98	2.73
0	0.106	433	42.5	3.31	42.5	3.31
60	0.106	433	31.2	2.67	36.5	2.99
240	0.106	433	12.6	1.43	24.5	2.29
360	0.106	433	7.08	0.97	19.5	1.99
600	0.106	433	2.65	0.51	13.6	1.62
0	0.198	433	17.1	3.39	17.1	3.39
60	0.198	433	11.7	2.55	14.3	2.95
240	0.198	433	4.06	1.16	9.11	2.14
360	0.198	433	2.26	0.76	7.27	1.84
600	0.198	433	1.11	0.51	5.27	1.52
0	0.291	433	3.87	3.45	3.87	3.45
60	0.291	433	4.09	2.46	3.98	2.94
240	0.291	433	3.71	1.04	4.04	2.07
360	0.291	433	2.80	0.72	3.94	1.79
600	0.291	433	1.75	0.62	3.65	1.52
0	0.383	433	1.06	3.51	1.06	3.51
60	0.383	433	7.79	2.41	2.38	2.94
240	0.383	433	5.38	0.98	3.94	2.04
360	0.383	433	2.92	0.74	3.94	1.77

Table M4. Continued

Pretreatment conditions			Selectivity			
Time	Pressure[(a)]	Temperature	Differential		Integral	
(min)	ratio	(K)	Glucan	Xylan	Glucan	Xylan
600	0.383	433	1.68	0.72	3.64	1.54
0	0.106	453	17.3	2.63	17.3	2.63
60	0.106	453	10.1	1.83	13.4	2.22
360	0.106	453	1.01	0.38	5.47	1.29
600	0.106	453	0.45	0.27	3.66	1.06
0	0.198	453	10.0	2.70	10.0	2.70
60	0.198	453	5.14	1.67	7.33	2.16
360	0.198	453	0.57	0.40	2.91	1.22
600	0.198	453	0.47	0.48	2.13	1.06
0	0.291	453	3.06	2.75	3.06	2.75
60	0.291	453	3.05	1.56	3.13	2.13
240	0.291	453	1.07	0.52	2.61	1.37
360	0.291	453	0.73	0.49	2.28	1.22
600	0.291	453	0.60	0.63	1.87	1.10
0	0.383	453	0.91	2.80	0.91	2.80
60	0.383	453	5.69	1.50	2.61	2.11
360	0.383	453	0.73	0.59	2.20	1.24
600	0.383	453	0.57	0.68	1.81	1.14
600	0.383	433	1.68	0.72	3.64	1.54

(a) Ratio kg oxygen/kg biomass

APPENDIX N

ALL DATA IN SECTION SELECTIVITY AND DELIGNIFICATION KINETICS FOR OXIDATIVE AND NON-OXIDATIVE LIME PRETREATMENT OF POPLAR WOOD. PART III: LONG-TERM

Table N1. Lignin measured vs. lignin calculated and model assessment for Model 1 and all pretreatment conditions (α=0.05).

Pretreatment conditions			Measured lignin yield	Calculated			Model assessment	
Time (wks)	Temp (K)	Air[a]		Fast degrading lignin	Slow degrading lignin	Lignin total	Residuals	Y_L CI[b]
0	298	1	1.000	0.235	0.765	1.000	0.000	0.000
1	298	1	0.880	0.102	0.732	0.891	-0.011	0.015
2	298	1	0.840	0.045	0.700	0.818	0.022	0.018
4	298	1	0.720	0.008	0.640	0.730	-0.010	0.014
7	298	1	0.650	0.001	0.560	0.658	-0.008	0.011
8	298	1	0.632	0.000	0.536	0.640	-0.008	0.011
12	298	1	0.569	0.000	0.448	0.579	-0.010	0.016
0	308	1	1.000	0.235	0.765	1.000	0.000	0.000
1	308	1	0.841	0.123	0.741	0.864	-0.023	0.012
2	308	1	0.800	0.065	0.717	0.781	0.019	0.011
4	308	1	0.700	0.018	0.672	0.689	0.011	0.009
7	308	1	0.610	0.003	0.609	0.612	-0.002	0.010
8	308	1	0.580	0.001	0.590	0.591	-0.011	0.010
12	308	1	0.560	0.000	0.518	0.518	0.042	0.015
0	318	1	1.000	0.235	0.765	1.000	0.000	0.000
1	318	1	0.830	0.102	0.732	0.834	-0.004	0.013
2	318	1	0.740	0.045	0.700	0.744	-0.004	0.010
4	318	1	0.650	0.008	0.640	0.649	0.001	0.010
7	318	1	0.560	0.001	0.560	0.561	-0.001	0.008
8	318	1	0.520	0.000	0.536	0.536	-0.016	0.008
12	318	1	0.439	0.000	0.448	0.448	-0.009	0.014
0	328	1	1.000	0.235	0.765	1.000	0.000	0.000
1	328	1	0.800	0.082	0.721	0.802	-0.002	0.017
2	328	1	0.730	0.029	0.679	0.707	0.023	0.011
4	328	1	0.620	0.003	0.602	0.606	0.014	0.012
7	328	1	0.518	0.000	0.503	0.503	0.014	0.009
8	328	1	0.450	0.000	0.474	0.474	-0.024	0.009

Table N1. Continued

Pretreatment conditions			Measured lignin yield	Calculated			Model assessment	
Time (wks)	Temp (K)	Air[(a)]		Fast degrading lignin	Slow degrading lignin	Lignin total	Residuals	Y_L CI[(b)]
12	328	1	0.380	0.000	0.373	0.373	0.007	0.014
0	338	1	1.000	0.235	0.765	1.000	0.000	0.000
1	338	1	0.750	0.063	0.707	0.770	-0.020	0.020
2	338	1	0.668	0.017	0.653	0.670	-0.002	0.014
4	338	1	0.544	0.001	0.558	0.559	-0.015	0.015
7	338	1	0.470	0.000	0.440	0.440	0.030	0.013
8	338	1	0.400	0.000	0.407	0.407	-0.007	0.014
12	338	1	0.287	0.000	0.296	0.296	-0.009	0.017
0	298	2	1.000	0.209	0.791	1.000	0.000	0.000
1	298	2	0.920	0.144	0.790	0.934	-0.014	0.023
2	298	2	0.880	0.099	0.789	0.888	-0.008	0.029
4	298	2	0.848	0.047	0.787	0.834	0.013	0.023
7	298	2	0.824	0.015	0.784	0.800	0.025	0.015
8	298	2	0.800	0.011	0.783	0.794	0.006	0.015
12	298	2	0.750	0.002	0.780	0.782	-0.032	0.020
0	308	2	1.000	0.209	0.791	1.000	0.000	0.000
1	308	2	0.880	0.115	0.789	0.904	-0.024	0.021
2	308	2	0.846	0.063	0.788	0.851	-0.004	0.018
4	308	2	0.834	0.019	0.784	0.803	0.031	0.013
7	308	2	0.790	0.003	0.779	0.782	0.009	0.014
8	308	2	0.732	0.002	0.777	0.779	-0.046	0.015
12	308	2	0.700	0.000	0.770	0.770	-0.070	0.022
0	318	2	1.000	0.209	0.791	1.000	0.000	0.000
1	318	2	0.850	0.082	0.788	0.870	-0.020	0.026
2	318	2	0.830	0.032	0.785	0.817	0.013	0.015
4	318	2	0.800	0.005	0.778	0.783	0.017	0.015
7	318	2	0.750	0.000	0.769	0.769	-0.019	0.012
8	318	2	0.754	0.000	0.766	0.766	-0.012	0.013
12	318	2	0.726	0.000	0.753	0.753	-0.027	0.023
0	328	2	1.000	0.209	0.791	1.000	0.000	0.000
1	328	2	0.850	0.051	0.786	0.836	-0.036	0.029
2	328	2	0.830	0.012	0.780	0.792	-0.012	0.017
4	328	2	0.800	0.001	0.769	0.770	-0.020	0.016
7	328	2	0.750	0.000	0.753	0.753	-0.053	0.012
8	328	2	0.754	0.000	0.748	0.748	-0.068	0.013
12	328	2	0.726	0.000	0.727	0.727	-0.067	0.023
0	338	2	1.000	0.209	0.791	1.000	0.000	0.000
1	338	2	0.800	0.026	0.782	0.808	-0.008	0.024

Table N1. Continued

Pretreatment conditions			Measured lignin yield	Calculated			Model assessment	
Time (wks)	Temp (K)	Air[a]		Fast degrading lignin	Slow degrading lignin	Lignin total	Residuals	Y_L CI[b]
2	338	2	0.760	0.003	0.773	0.776	-0.016	0.023
4	338	2	0.700	0.000	0.755	0.755	-0.055	0.020
7	338	2	0.680	0.000	0.729	0.729	-0.049	0.021
8	338	2	0.689	0.000	0.720	0.720	-0.031	0.023
12	338	2	0.600	0.000	0.687	0.687	-0.087	0.033
2	338	2	0.760	0.003	0.773	0.776	-0.016	0.023
4	338	2	0.700	0.000	0.755	0.755	-0.055	0.020

(a) 1 with air, 2 without air.
(b) Confidence intervals for response variable.

Table N2. Glucan measured vs. glucan calculated and model assessment for Model 1 and all pretreatment conditions (α=0.05).

Pretreatment conditions			Measured glucan yield	Calculated			Model assessment	
Time (wks)	Temp (K)	Air[a]		Fast degrading glucan	Slow degrading glucan	Glucan total	Residuals	Y_L CI[b]
0	298	1	1.000	0.544	0.456	1.000	0.000	0.000
1	298	1	0.990	0.534	0.456	0.989	-0.001	0.003
2	298	1	0.990	0.523	0.456	0.979	-0.011	0.006
4	298	1	0.980	0.503	0.456	0.959	-0.021	0.012
7	298	1	0.950	0.474	0.456	0.930	-0.020	0.020
8	298	1	0.900	0.465	0.456	0.921	0.021	0.023
12	298	1	0.890	0.430	0.456	0.886	-0.004	0.032
0	308	1	1.000	0.544	0.456	1.000	0.000	0.000
1	308	1	1.004	0.527	0.456	0.983	-0.021	0.003
2	308	1	0.996	0.511	0.456	0.967	-0.029	0.007
4	308	1	0.989	0.479	0.456	0.935	-0.054	0.012
7	308	1	0.893	0.436	0.456	0.892	-0.002	0.020
8	308	1	0.830	0.422	0.456	0.878	0.048	0.022
12	308	1	0.822	0.372	0.456	0.828	0.006	0.030
0	318	1	1.000	0.544	0.456	1.000	0.000	0.000
1	318	1	0.965	0.518	0.456	0.974	0.009	0.004
2	318	1	0.930	0.493	0.456	0.948	0.018	0.007
4	318	1	0.900	0.446	0.456	0.902	0.002	0.012
7	318	1	0.846	0.384	0.456	0.840	-0.007	0.016
8	318	1	0.830	0.365	0.456	0.821	-0.009	0.018
12	318	1	0.800	0.299	0.456	0.755	-0.045	0.023
0	328	1	1.000	0.544	0.456	1.000	0.000	0.000
1	328	1	0.950	0.504	0.456	0.960	0.010	0.006
2	328	1	0.900	0.467	0.456	0.923	0.023	0.011
4	328	1	0.848	0.401	0.456	0.857	0.010	0.016
7	328	1	0.714	0.320	0.456	0.775	0.061	0.016
8	328	1	0.726	0.296	0.456	0.752	0.025	0.015
12	328	1	0.665	0.218	0.456	0.674	0.009	0.022
0	338	1	1.000	0.544	0.456	1.000	0.000	0.000
1	338	1	0.930	0.486	0.456	0.942	0.012	0.013
2	338	1	0.900	0.434	0.456	0.890	-0.010	0.021
4	338	1	0.856	0.346	0.456	0.802	-0.054	0.027
7	338	1	0.721	0.246	0.456	0.702	-0.019	0.025
8	338	1	0.664	0.220	0.456	0.676	0.012	0.024
12	338	1	0.600	0.140	0.456	0.596	-0.004	0.042

Table N2. Continued

Pretreatment conditions			Measured glucan yield	Calculated			Model assessment	
Time (wks)	Temp (K)	Air[a]		Fast degrading glucan	Slow degrading glucan	Glucan total	Residuals	Y_L CI[b]
0	298	2	1.000	0.013	0.987	1.000	0.000	0.000
1	298	2	0.990	0.012	0.985	0.997	0.007	0.032
2	298	2	0.990	0.012	0.982	0.994	0.004	0.061
4	298	2	0.990	0.012	0.976	0.988	-0.002	0.112
7	298	2	0.970	0.011	0.968	0.979	0.009	0.174
8	298	2	0.960	0.011	0.965	0.976	0.016	0.191
12	298	2	0.960	0.010	0.954	0.965	0.005	0.247
0	308	2	1.000	0.013	0.987	1.000	0.000	0.000
1	308	2	0.990	0.012	0.982	0.994	0.004	0.029
2	308	2	0.990	0.012	0.977	0.989	-0.001	0.055
4	308	2	0.990	0.012	0.966	0.978	-0.012	0.102
7	308	2	0.965	0.011	0.951	0.961	-0.003	0.160
8	308	2	0.956	0.011	0.945	0.956	0.000	0.176
12	308	2	0.932	0.010	0.925	0.935	0.002	0.226
0	318	2	1.000	0.013	0.987	1.000	0.000	0.000
1	318	2	0.990	0.012	0.978	0.990	0.000	0.022
2	318	2	0.990	0.012	0.968	0.980	-0.010	0.043
4	318	2	0.950	0.011	0.949	0.960	0.010	0.081
7	318	2	0.930	0.010	0.921	0.931	0.001	0.127
8	318	2	0.900	0.010	0.912	0.921	0.021	0.140
12	318	2	0.900	0.009	0.876	0.885	-0.015	0.182
0	328	2	1.000	0.013	0.987	1.000	0.000	0.000
1	328	2	0.990	0.012	0.970	0.982	-0.008	0.016
2	328	2	0.990	0.012	0.953	0.965	-0.025	0.030
4	328	2	0.946	0.011	0.920	0.930	-0.015	0.057
7	328	2	0.933	0.009	0.872	0.882	-0.051	0.093
8	328	2	0.872	0.009	0.857	0.866	-0.006	0.104
12	328	2	0.795	0.008	0.798	0.806	0.011	0.140
0	338	2	1.000	0.013	0.987	1.000	0.000	0.000
1	338	2	0.940	0.012	0.958	0.970	0.030	0.012
2	338	2	0.940	0.011	0.929	0.940	0.000	0.024
4	338	2	0.900	0.010	0.874	0.884	-0.016	0.047
7	338	2	0.780	0.009	0.798	0.807	0.027	0.080
8	338	2	0.774	0.008	0.774	0.782	0.008	0.091
12	338	2	0.700	0.006	0.685	0.692	-0.008	0.131

(a) 1 with air, 2 without air.

(b) Confidence intervals for response variable.

Table N3. Xylan measured vs. glucan calculated and model assessment for Model 1 and all pretreatment conditions (α=0.05).

Pretreatment conditions			Measured xylan yield	Calculated			Model assessment	
Time (wks)	Temp (K)	Air[(a)]		Fast degrading xylan	Slow degrading xylan	Xylan total	Residuals	Y_L CI[(b)]
0	298	1	1.000	0.111	0.889	1.000	0.000	0.000
1	298	1	0.882	0.070	0.851	0.921	-0.038	0.018
2	298	1	0.890	0.044	0.814	0.858	0.032	0.024
4	298	1	0.769	0.017	0.746	0.763	0.006	0.022
7	298	1	0.690	0.004	0.654	0.658	0.032	0.017
8	298	1	0.626	0.003	0.626	0.628	-0.002	0.018
12	298	1	0.532	0.000	0.525	0.525	0.007	0.025
0	308	1	1.000	0.111	0.889	1.000	0.000	0.000
1	308	1	0.933	0.059	0.845	0.905	0.029	0.017
2	308	1	0.873	0.032	0.804	0.836	0.038	0.017
4	308	1	0.730	0.009	0.727	0.736	-0.006	0.012
7	308	1	0.600	0.001	0.626	0.627	-0.028	0.014
8	308	1	0.600	0.001	0.595	0.596	0.004	0.015
12	308	1	0.500	0.000	0.487	0.487	0.013	0.020
0	318	1	1.000	0.111	0.889	1.000	0.000	0.000
1	318	1	0.838	0.048	0.840	0.888	-0.050	0.022
2	318	1	0.800	0.021	0.793	0.815	-0.015	0.015
4	318	1	0.717	0.004	0.708	0.712	0.005	0.014
7	318	1	0.650	0.000	0.597	0.597	0.053	0.012
8	318	1	0.554	0.000	0.564	0.564	-0.011	0.012
12	318	1	0.431	0.000	0.449	0.449	-0.018	0.017
0	328	1	1.000	0.111	0.889	1.000	0.000	0.000
1	328	1	0.879	0.038	0.834	0.872	0.008	0.028
2	328	1	0.780	0.013	0.782	0.795	-0.015	0.017
4	328	1	0.671	0.001	0.688	0.690	-0.019	0.018
7	328	1	0.580	0.000	0.568	0.568	0.012	0.013
8	328	1	0.522	0.000	0.533	0.533	-0.011	0.013
12	328	1	0.400	0.000	0.413	0.413	-0.013	0.020
0	338	1	1.000	0.111	0.889	1.000	0.000	0.000
1	338	1	0.869	0.028	0.828	0.855	0.013	0.027
2	338	1	0.750	0.007	0.771	0.778	-0.028	0.024
4	338	1	0.700	0.000	0.668	0.668	0.032	0.020
7	338	1	0.560	0.000	0.539	0.539	0.021	0.018
8	338	1	0.500	0.000	0.502	0.502	-0.002	0.020
12	338	1	0.380	0.000	0.377	0.377	0.003	0.026

Table N3. Continued

Pretreatment conditions			Measured xylan yield	Calculated			Model assessment	
Time (wks)	Temp (K)	Air[a]		Fast degrading xylan	Slow degrading xylan	Xylan total	Residuals	Y_L CI[b]
0	298	2	1.000	0.065	0.935	1.000	0.000	0.000
1	298	2	0.897	0.000	0.913	0.913	0.017	0.017
2	298	2	0.900	0.000	0.892	0.892	-0.008	0.016
4	298	2	0.852	0.000	0.851	0.851	-0.001	0.015
7	298	2	0.794	0.000	0.792	0.792	-0.002	0.016
8	298	2	0.789	0.000	0.773	0.773	-0.015	0.018
12	298	2	0.678	0.000	0.703	0.703	0.026	0.024
0	308	2	1.000	0.065	0.935	1.000	0.000	0.000
1	308	2	0.908	0.000	0.909	0.909	0.001	0.017
2	308	2	0.890	0.000	0.884	0.884	-0.006	0.015
4	308	2	0.839	0.000	0.835	0.835	-0.004	0.013
7	308	2	0.795	0.000	0.767	0.767	-0.028	0.014
8	308	2	0.736	0.000	0.746	0.746	0.010	0.015
12	308	2	0.650	0.000	0.666	0.666	0.016	0.019
0	318	2	1.000	0.065	0.935	1.000	0.000	0.000
1	318	2	0.906	0.000	0.905	0.905	-0.002	0.017
2	318	2	0.880	0.000	0.875	0.875	-0.005	0.015
4	318	2	0.867	0.000	0.818	0.818	-0.049	0.012
7	318	2	0.770	0.000	0.740	0.740	-0.030	0.012
8	318	2	0.730	0.000	0.716	0.716	-0.014	0.013
12	318	2	0.590	0.000	0.626	0.626	0.036	0.016
0	328	2	1.000	0.065	0.935	1.000	0.000	0.000
1	328	2	0.850	0.000	0.900	0.900	0.050	0.017
2	328	2	0.840	0.000	0.865	0.865	0.025	0.015
4	328	2	0.800	0.000	0.800	0.800	0.000	0.012
7	328	2	0.730	0.000	0.712	0.712	-0.018	0.013
8	328	2	0.680	0.000	0.685	0.685	0.005	0.014
12	328	2	0.600	0.000	0.586	0.586	-0.014	0.018
0	338	2	1.000	0.065	0.935	1.000	0.000	0.000
1	338	2	0.880	0.000	0.894	0.894	0.014	0.017
2	338	2	0.850	0.000	0.855	0.855	0.005	0.015
4	338	2	0.800	0.000	0.781	0.781	-0.019	0.014
7	338	2	0.700	0.000	0.682	0.682	-0.018	0.018
8	338	2	0.660	0.000	0.652	0.652	-0.008	0.019
12	338	2	0.504	0.000	0.544	0.544	0.040	0.024

(a) 1 with air, 2 without air.
(b) Confidence intervals for response variable.

Table N4. Differential and integral selectivity

Pretreatment conditions			Selectivity			
Time (wks)	Air[(a)]	Temperature (K)	Differential		Integral	
			Glucan	Xylan	Glucan	Xylan
0	1	298	12.747	1.512	12.747	1.512
1	1	298	8.496	1.284	10.458	1.401
2	1	298	5.871	1.082	8.787	1.306
4	1	298	3.247	0.792	6.617	1.156
7	1	298	2.001	0.612	4.916	1.012
8	1	298	1.851	0.593	4.567	0.979
12	1	298	1.629	0.595	3.689	0.894
0	1	308	10.410	1.583	10.410	1.583
1	1	308	6.232	1.318	8.115	1.458
2	1	308	3.999	1.084	6.585	1.351
4	1	308	2.166	0.788	4.804	1.191
7	1	308	1.544	0.667	3.585	1.050
8	1	308	1.491	0.664	3.354	1.020
12	1	308	1.432	0.694	2.799	0.947
0	1	318	8.615	1.643	8.615	1.643
1	1	318	4.575	1.350	6.345	1.509
2	1	318	2.760	1.090	4.985	1.395
4	1	318	1.585	0.815	3.581	1.231
7	1	318	1.331	0.750	2.743	1.099
8	1	318	1.323	0.753	2.595	1.073
12	1	318	1.340	0.785	2.254	1.009
0	1	328	7.215	1.694	7.215	1.694
1	1	328	3.372	1.384	5.001	1.559
2	1	328	1.969	1.106	3.828	1.443
4	1	328	1.291	0.872	2.765	1.281
7	1	328	1.244	0.836	2.211	1.159
8	1	328	1.261	0.838	2.121	1.134
12	1	328	1.344	0.848	1.925	1.074
0	1	338	6.109	1.737	6.109	1.737
1	1	338	2.510	1.425	3.979	1.611
2	1	338	1.486	1.139	2.999	1.496
4	1	338	1.161	0.950	2.225	1.340
7	1	338	1.248	0.908	1.879	1.223
8	1	338	1.291	0.900	1.830	1.200
12	1	338	1.482	0.870	1.740	1.137

Table N4. Continued

Pretreatment conditions			Selectivity			
Time (wks)	Air[(a)]	Temperature (K)	Differential		Integral	
			Glucan	Xylan	Glucan	Xylan
0	2	298	26.655	1.853	26.655	1.853
1	2	298	18.349	1.397	22.239	1.618
2	2	298	12.690	1.059	18.797	1.429
4	2	298	6.209	0.621	13.938	1.156
7	2	298	2.371	0.311	9.682	0.909
8	2	298	1.806	0.259	8.743	0.854
12	2	298	0.860	0.176	6.271	0.712
0	2	308	22.794	2.634	22.794	2.634
1	2	308	12.584	1.604	17.174	2.083
2	2	308	7.069	0.994	13.373	1.702
4	2	308	2.481	0.424	8.875	1.239
7	2	308	0.891	0.203	5.735	0.912
8	2	308	0.755	0.189	5.133	0.851
12	2	308	0.612	0.222	3.678	0.714
0	2	318	19.604	3.630	19.604	3.630
1	2	318	7.876	1.616	12.827	2.500
2	2	318	3.379	0.768	9.072	1.858
4	2	318	0.995	0.277	5.491	1.237
7	2	318	0.613	0.229	3.491	0.899
8	2	318	0.600	0.247	3.143	0.844
12	2	318	0.599	0.356	2.331	0.732
0	2	328	16.971	4.848	16.971	4.848
1	2	328	4.413	1.398	9.242	2.778
2	2	328	1.474	0.517	5.953	1.880
4	2	328	0.629	0.269	3.459	1.201
7	2	328	0.590	0.336	2.277	0.900
8	2	328	0.593	0.370	2.079	0.855
12	2	328	0.606	0.530	1.621	0.772
0	2	338	14.795	6.260	14.795	6.260
1	2	338	2.232	1.041	6.453	2.865
2	2	338	0.764	0.392	3.895	1.811
4	2	338	0.582	0.358	2.307	1.170
7	2	338	0.596	0.473	1.615	0.920
8	2	338	0.601	0.517	1.501	0.886
12	2	338	0.624	0.709	1.240	0.830

(a) 1 with air, 2 without air.

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